

# Investigation of potential diseases associated with Northern Territory mammal declines | Final report

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**Murdoch**  
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Government**

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Photos in the report were taken by A Reiss, S Young, T Portas and M Lawrence-Taylor. Map courtesy of Aaron Gove.

# Contents

Executive summary.....	1
1 Introduction.....	3
1.1 Declining small mammal populations in the Top End of the Northern Territory ...	3
1.2 The importance of disease in wildlife and declining populations.....	4
1.2.1 Emerging infectious diseases .....	6
1.2.2 Challenges in investigating disease in free-ranging wildlife populations ....	6
1.3 Understanding disease processes.....	8
1.4 General indicators of health and disease .....	8
1.4.1 Macroparasites.....	9
1.5 Disease in small mammal species in Australia (with a focus on the tropical north)..	9
1.6 Aims of this project.....	10
2 Materials and methods .....	11
2.1 Selection of species and sites .....	11
2.2 Development of protocols .....	11
2.3 Review of information on disease in target species and in the Top End .....	11
2.4 Hazard identification and assessment.....	12
2.4.1 Hazard scoring and prioritisation .....	12
2.5 Field processes.....	13
2.5.1 Trapping .....	13
2.5.2 Anaesthesia .....	15
2.5.3 Morphometric and demographic measurements and health assessments .	16
2.5.4 Blood and other specimen sampling .....	18
2.5.5 Ectoparasites and skin disease investigation .....	20
2.5.6 Management of samples in the field .....	21
2.5.7 Post mortem sampling.....	21
2.6 Laboratory testing of samples .....	21
2.6.1 Pathogen testing .....	23
2.6.2 Haematological and biochemical testing.....	26
2.7 Data management and statistical analyses .....	27
2.7.1 Descriptive analyses .....	27
2.7.2 Univariate analyses .....	28

3	Results .....	29
3.1	Hazard identification and prioritisation .....	29
3.2	Wildlife Health Australia and Australian Registry of Wildlife Health data .....	32
3.3	Existing knowledge of disease in target taxa .....	32
	3.3.1 Dasyurids and northern quolls .....	33
	3.3.2 Bandicoots and northern brown bandicoots .....	34
	3.3.3 Phalangeridae and brushtail possums .....	36
3.4	Significant diseases and their potential impact on small mammals in the Top End ..	39
	3.4.1 Viruses .....	39
	3.4.2 Bacteria .....	41
	3.4.3 Protozoa .....	43
	3.4.4 Helminth endoparasites .....	47
	3.4.5 Ectoparasites .....	48
3.5	Ancillary tests and investigations of relevance to our study .....	49
	3.5.1 Haematological analyses .....	49
	3.5.2 Biochemical analyses .....	49
	3.5.3 <i>De novo</i> pathogen sequencing .....	49
	3.5.4 Stress studies .....	50
	3.5.5 Studies of anti-oxidant capacity .....	50
3.6	Trapping and sampling .....	50
3.7	Health assessment .....	51
	3.7.1 Health status by clinical examination .....	51
	3.7.2 Body condition and weights .....	53
	3.7.3 Skin disease and ectoparasites .....	58
	3.7.4 Haematological and biochemical indicators of health status .....	62
3.8	Pathogen testing .....	68
	3.8.1 Direct evidence of pathogen presence .....	70
	3.8.2 Serological evidence of exposure to pathogens .....	78
	3.8.3 Repeat sampling .....	81
3.9	Statistically significant variables .....	81

4	Discussion.....	82
4.1	Health assessments and demographics .....	83
4.2	Body condition and weights .....	83
4.3	Skin and ectoparasites.....	84
4.4	Haematology and biochemistry variables .....	85
4.5	Interpretation of pathogen tests.....	86
4.6	Direct evidence of pathogen presence.....	87
4.6.1	Herpesvirus.....	87
4.6.2	<i>Salmonella</i> spp. ....	88
4.6.3	<i>Babesia</i> spp. and <i>Hepatozoon</i> spp. ....	90
4.6.4	Trypanosomes .....	90
4.6.5	Protozoal endoparasites.....	91
4.6.6	Microfilaria.....	92
4.6.7	Helminth endoparasites .....	92
4.6.8	<i>De novo</i> molecular testing .....	93
4.7	Serological evidence of exposure to pathogens .....	93
4.7.1	Barmah Forest virus and Ross River virus .....	93
4.7.2	Encephalomyocarditis virus .....	93
4.7.3	<i>Coxiella burnetii</i> (Q fever).....	94
4.7.4	<i>Leptospira</i> spp.....	95
4.7.5	<i>Toxoplasma gondii</i> .....	96
4.8	Repeat testing.....	98
4.9	Resilience and susceptibility of Top End populations to disease .....	99
4.10	Project limitations.....	100
4.11	Additional project outcomes .....	100
5	Conclusion.....	101
5.1	Is disease a factor in declines of small mammals in the Top End?.....	101
5.2	What do we recommend? .....	102
5.2.1	High priority recommendations.....	102
5.2.2	Medium priority recommendations.....	103
5.2.3	Lower priority recommendations .....	104

Appendix 1 - Infection, disease, diagnostic testing and interpretation .....	105
Infection and disease .....	105
Testing for disease .....	107
Sensitivity and specificity of tests .....	107
Tests for direct evidence of pathogens.....	108
Tests for indirect evidence of pathogens .....	108
Interpretation of test results .....	109
Appendix 2 - NERP - Small Mammal Disease Investigation protocols.....	110
Appendix 3 - NERP - Small Mammal Disease Investigation field data sheet.....	111
Appendix 4 - Further information on pathogens not prioritised for investigation.....	113
Notes on other bacteria not prioritised for investigation.....	113
Notes on fungi not prioritised for investigation.....	115
Notes on other protozoa not prioritised for investigation .....	115
Appendix 5 - Scientific presentations and media interactions.....	117



## Glossary and abbreviations used in this report

ANOVA	Analysis of variance
Antibody	A blood protein produced by the body's immune system in response to a disease; antibodies can often be detected in an animal long after recovery from the disease
ARWH	Australian Registry of Wildlife Health
Bacterium	A type of microscopic organism that can cause disease; examples of bacterial disease include cholera and bubonic plague ( <i>p/l. bacteria</i> )
BVL	Berrimah Veterinary Laboratories, NT
Cestode	A group of helminth parasites; also known as tapeworm
CFT	Complement fixation test (a type of serological test for antibodies)
CNS	Central nervous system
Disease	Any disturbance in the health or function of an animal
DLRM	Department of Land Resource Management, NT Government
DNA	Deoxyribonucleic acid
ECMV	Encephalomyocarditis virus
Ectoparasite	Parasite living outside the host's body e.g., ticks, fleas and mites
EDTA	Ethylenediaminetetraacetic acid; used to prevent clotting of blood samples after collection
ELISA	Enzyme-linked immunosorbent assay (a type of serological test for antibodies)
EMAI	Elizabeth MacArthur Agricultural Institute, NSW
Endoparasite	Parasite occurring inside the host's body e.g., worms in the gut
Exotic	Not currently present in Australia
FEC	Faecal egg count
GIT	Gastrointestinal tract
GLM	Generalised linear models
Helminth	The group name for all parasitic worms
Host	An animal that carries a parasite, virus or disease
HV	Herpesvirus
IM	Intra-muscular (injection)
Lesion	Any abnormality or damage in a tissue or organ of the body

Macroparasite	Parasites such as fleas, ticks, mites and worms (nematodes, trematodes, flukes), as compared to microparasites which includes bacteria and viruses; may be endoparasites or ectoparasites
MAT	Modified agglutination test (a type of serological test for antibodies)
Nematode	A group of helminth parasites; also known as round worms
Neoplasia	Cancer; malignant growth or disease
NERP	National Environmental Research Program
NT	Northern Territory
NZ	New Zealand
Parasite	Organism that lives in or on another organism (the host) and causes it harm; may be virus, bacteria, insect, worms, etc.
Pathogen	Any infectious agent capable of causing disease in a host, e.g., viruses, bacteria, macroparasites, protozoa, fungi
PCR	Polymerase chain reaction; a test used to amplify and identify specific pieces of DNA; it can detect DNA from a disease agent in very small volumes
PCV	Packed cell volume of blood; one of the standard health tests performed on blood samples; the relative volume of blood cells to blood serum
Protozoa	Single-celled organisms that can be found as parasites in animals; some are known to cause disease, such as malaria or <i>Giardia</i> spp.
PY	Pouch young
QF	Q fever ( <i>Coxiella burnetii</i> )
Qld	Queensland
RNA	Ribonucleic acid
Serovar	A subdivision or strain of a species of pathogen
Tas	Tasmania
TPP	Total plasma protein; one of the standard health tests performed to measure the total amount of protein in a blood sample
Trematode	A group of helminth parasites; also known as flukes
Vector	A carrier that transmits a disease from one organism to another
Virus	A type of microscopic organism that can cause disease; viruses need to use their host's cells to replicate; examples include the common cold and influenza
VNT	Virus neutralisation test (a type of serological test for antibodies)
WA	Western Australia
WHA	Wildlife Health Australia

## Executive summary

There is compelling evidence of broad-scale declines in populations of small terrestrial native mammals in northern Australia, including the Top End of the Northern Territory (NT) over the past 20 years. Causes under consideration include changed fire regimes, introduced fauna (including predators) and disease. To date information on health and disease in northern Australian mammals has been limited.

Disease is increasingly recognised as a primary driver of some wildlife population declines and extinctions e.g., Tasmanian devil facial tumour disease, white nose syndrome in bats and chytrid fungus in amphibians.

Disease has been identified as a risk factor for extinction in declining and fragmented wildlife populations globally, particularly in situations of increased environmental stressors, changing ecosystems, arrival of new vertebrate threats or climate change. Unless wild populations are studied in detail over long periods of time, the effects of disease are easily overlooked and may be difficult to determine.

This study is the largest and most comprehensive study of health and disease in small mammals in northern Australia and is one of a small number of studies worldwide to have approached investigation of wildlife populations in this comprehensive manner.

A total of 281 individuals from four target species were examined and sampled under anaesthesia across five main sites in the Top End of the NT, from June 2013 to Nov 2014. Non-invasive samples (ticks and faeces) were collected from a further 113 animals.

Nine prioritised pathogen groups were investigated by diagnostic testing:

- encephalomyocarditis virus (EMCV)
- mammalian herpesvirus
- *Coxiella burnetii* (disease agent causing Q fever)
- *Leptospira* spp.
- enteric *Salmonella* spp.
- enteric protozoa (*Cryptosporidium* spp. and *Giardia* spp.)
- protozoal haemoparasites (trypanosomes, *Babesia* spp. and *Hepatozoon* spp.)
- *Toxoplasma gondii*
- gastrointestinal helminths (worms)

Additional investigation was undertaken under collaborative agreements for pathogens of significance to human health: Ross River virus and Barmah Forest virus.

Results were analysed for associations with locations, species, seasons, body condition, sex, blood parameters and other potential health indicators such as level of ectoparasite burden.

The majority of individuals examined were assessed to be in good health and body condition.

The presence of several pathogens which are known to be associated with disease in wildlife populations was identified, including mammalian herpesvirus, enteric *Salmonella* spp., protozoal haemoparasites (trypanosomes, *Babesia* spp., *Hepatozoon* spp.), enteric protozoa (*Cryptosporidium* spp. and *Giardia* spp.), microfilaria and *Toxoplasma*. Of these, several were previously unreported in target species in the NT.

*De novo* molecular pathogen discovery studies on the northern brown bandicoot (*Isodon macrourus*) used cutting-edge metagenomic techniques to look for unrecognised pathogens. Analysis is still underway; however there is evidence of several potentially significant pathogens including viruses from the Retroviridae family.

A number of ectoparasite taxa that may act as vectors for infectious disease were identified.

The study found evidence that several pathogens, capable of impacting population health, are circulating in Top End small mammal populations, but did not find compelling evidence that a single pathogen is responsible for, or a risk factor in, the decline of small mammals in the Top End of the NT.

The study found no serological evidence of infection with encephalomyocarditis virus, *Leptospira* spp. or *Coxiella burnetii*; however it is possible that these pathogens are present in populations (at levels below detection limits due to sample sizes), with resultant morbidity or mortality.

Limitations of this study include a short temporal span, a lack of longitudinal and survivorship studies, difficulties in collecting specimens from small species and an inability to study populations in the absence of feral cats and other predators. In combination these factors limit the ability to investigate the potentially complex interactions between disease and other pressures on mammal populations.

Top End mammal populations are assessed to be at risk of increased levels of environmental and host stresses and are vulnerable to the likely future impacts of infectious disease. The current situation in the Top End fulfils many of the criteria necessary for an emerging infectious disease and novel disease agents should be considered as risk factors for populations. It is recommended that disease investigation continues in mammal populations of concern in the Top End in the medium to long term (five to 20 years) to increase knowledge; improve data sets; maintain and build capacity; and maximise opportunities for detection and response to new disease threats.

It is recommended that future studies include, as priorities:

- longitudinal and survivorship studies to determine the impact of nominated pathogens on host survival and fitness (e.g., using cortisol and anti-oxidant capacity), and detect pathogen trends in individuals (e.g. whether certain pathogens are shed intermittently and whether infections persist or resolve).
- a particular focus on *Toxoplasma gondii* (due to its strong epidemiological link to presence of cats in the environment and as the only identified pathogen likely to impact a broad taxonomic range of mammals) and any potentially significant pathogens emerging from *de novo* molecular work. This should include a focus on sites where feral cats have been excluded, to investigate potential interactions between pathogen presence and predation as well as differences in prevalence of *T. gondii* in areas where the definitive host (the cat) is present/absent
- extension of serological studies to a wider host species range (including macropods) to determine the presence and prevalence of priority pathogens such as *T. gondii* in the landscape.

Ongoing collaborative efforts between NT Department of Land Resource Management and the Conservation Medicine Program (School of Veterinary and Life Sciences), Murdoch University may facilitate research and enable continued opportunities for work funded through competitive grants or industry sponsorship.

# 1 Introduction

## 1.1 Declining small mammal populations in the Top End of the Northern Territory

There is compelling evidence of broad-scale declines in populations of small terrestrial native mammals across the monsoonal tropics of the Northern Territory (NT) of Australia, commonly referred to as the Top End (Fitzsimons *et al.*, 2010, Woinarski *et al.*, 2010, Woinarski *et al.*, 2012, Ziembicki *et al.*, 2015). Similar declines also appear to be underway in the tropical north of Queensland (Qld) and the tropical regions of Western Australia (WA) (Legge *et al.*, 2011, Woinarski *et al.*, 2011, Woinarski *et al.*, 2012, Ziembicki *et al.*, 2015). Previously, this area of Australia was considered to be relatively secure from ecological threats (Ziembicki *et al.*, 2013). The potential causes of the decline in the Top End are currently under investigation.

The tropical north of Australia is a vast, sparsely populated area with few centres of urbanisation. Much of the landscape remains in a relatively natural state, with large areas protected within national or private reserves. Limited studies over a large area have meant that the trends in small mammal population status have been difficult to accurately define (Woinarski *et al.*, 2011, Ziembicki *et al.*, 2015). Evidence for population declines in the monsoonal north of Australia comes from fauna monitoring programs; comparison of recent survey data with historical accounts and from consultation with indigenous land users in several regions of the Top End (Ziembicki *et al.*, 2013). Evidence to date strongly suggests that there has been a decline in both range and abundance of many species of small to medium sized terrestrial mammals, previously considered abundant or common, and that this decline has been occurring for more than 20 years (Woinarski *et al.*, 2015).

The declines have been documented across a variety of sites, including areas with apparently relatively undisturbed ecosystems and natural vegetation. In the Top End, populations of larger native mammal species, such as macropods, appear to be unaffected. Declines have occurred in species below 5 kg, with some very small sized species (<50 g) also in decline (Ziembicki *et al.*, 2015). The monsoonal north of Australia has been actively managed by the indigenous land users for many thousands of years; the most obvious management practice being the annual use of mosaic fires in the landscape to manage movement and harvest of food species. However in recent decades fire regimes have changed, with more intense, wide-spread and frequent burning now occurring (Russell-Smith *et al.*, 2003).

The reasons for population decline within relatively intact landscapes in northern Australia have not been easy to define and causes are not well understood. Factors that are considered to be important include:

- changing fire regimes
- introduced vegetation (in particular grasses)
- introduced predators (primarily feral cats)
- black rats (*Rattus rattus*) which may act competitively (as predators or vectors for infectious agents)
- disease
- grazing pressure on the landscape from introduced herbivores (mainly cattle, also water buffalo, feral pigs, horses, donkeys and banteng [*Bos javanicus*])
- arrival of the poisonous cane toad (*Rhinella marina*)

It is likely that some, if not all, of these factors are contributors to the mammalian population declines and factors may be acting synergistically (Ziembicki *et al.*, 2015). For example, introduced vegetation (in particular exotic grasses such as gamba grass [*Andropogon gayanus*]) create large fuel loads which promote 'hot' and widespread fires late in the dry season, in contrast to the 'cool', early dry season mosaic-type fires that were typically employed by indigenous land users (Russell-Smith *et al.*, 2003). The result may be a significant change in vegetation patterns and ecosystems over a relatively short period of time, which may allow introduced predators to undertake hunting in more effective ways (Ziembicki *et al.*, 2015). Likewise, disease (which may be a proximate or an ultimate factor) may be acting in a simplistic manner, or more plausibly, may be one factor in a multifactorial, perhaps synergistic process driving population declines. Disease should be considered as a possible factor in all declining wildlife populations (Wobeser, 2006), including those of mammals in northern Australia.

As summarised by Legge *et al.* (2011): 'Given the geographic and taxonomic spread of the native mammal declines in northern Australia, it is unlikely that any one of the key threats of changed fire, introduced herbivores, introduced predators, disease and exotic plant species is singularly responsible. The relative contribution of each threat probably varies depending on the species and location, and the compounded consequence of all threats may be to reduce the resilience of the system to cope with any additional impacts, no matter how minor...the threats almost certainly impact in a synergistic way.'

## 1.2 The importance of disease in wildlife and declining populations

Disease is increasingly recognised as a key threat to wildlife populations and to biodiversity in general, resulting in significant population declines and even extinction of wildlife species (Daszak *et al.*, 2000, de Castro and Bolker, 2005, Skerratt *et al.*, 2007, Grogan *et al.*, 2014). Given the lack of historic disease surveillance of wildlife populations and paucity of information in this area, it remains unknown how many wildlife extinctions have been caused by infection or as a result of the 'knock-on' effects of disease (Daszak and Cunningham, 1999).

A range of viruses, bacteria, fungi, protozoa, helminths and ectoparasites have been identified as posing an extinction risk to wild mammal species (Pedersen *et al.*, 2007). Rinderpest virus resulted in the death of more than 95% of wild ungulates in southern and eastern Africa when introduced in the 1890's (Daszak *et al.*, 2000). More recently, wildlife species that have suffered significant population declines associated with infectious disease include (but are not limited to) black-footed ferrets (*Mustela nigripes*; bacterial plague and canine distemper); amphibian species (fungal chytridiomycosis); lions (*Panthera leo*; canine distemper); micro bats (fungal white nose syndrome) and African wild dogs (*Lycaon pictus*; rabies and canine distemper) (Bengis *et al.*, 2004, Hofmeyr *et al.*, 2004, Skerratt *et al.*, 2007, Frick *et al.*, 2010).

Disease may act as a primary driver of population declines by directly increasing mortality. Devil facial tumour disease caused a 60% decline in species numbers of Tasmanian devils (*Sarcophilus harrisii*) within a 10 year period and chytridiomycosis infection has resulted in declines or extinctions in an estimated 200 amphibian species globally (Skerratt *et al.*, 2007, McCallum, 2008). Novel diseases can have devastating effects on highly susceptible and naïve populations. White nose syndrome in North America has resulted in a decline of up to 80% in some bat populations in Northern America, since its emergence in 2006 (Frick *et al.*, 2010).

Disease may drive population declines by reducing fecundity e.g., chlamydiosis in koalas (*Phascolarctos cinereus*) (Blanshard and Bodley, 2008, Patterson *et al.*, 2015). Disease may cause morbidity (ill health) which indirectly results in increased mortality. For example, Wallal virus, an orbivirus causing episodic outbreaks of disease in large macropods in inland Australia, results in blindness, which ultimately leads to starvation of affected animals (Hooper *et al.*, 1999, WHA, 2010). Disease may exert effects in more subtle, contributory manners e.g., immunological impacts in western ringtail possums (*Pseudocheirus occidentalis*) where survivorship of translocated animals was associated with changes in white cell counts (Clarke, 2011); and changes in behaviour of marsupials with toxoplasmosis, which resulted in increased docility and daytime activity (Hollings *et al.*, 2013). Such impacts may result in population level effects if, for example, behavioural changes result in increased risk of predation or motor vehicle trauma.

The interactive or synergistic effects of disease, alongside other ecological factors, are increasingly recognised in wildlife population declines. In many cases, the impacts of disease on wildlife are difficult to determine and may only be revealed through focused research over years or decades (Webster, 2007, Clarke, 2011, Miller *et al.*, 2012, Hollings *et al.*, 2013). The decline of the red squirrel (*Sciurus vulgaris*) in the UK was thought for many years to be due to competition from the newly arrived grey squirrel (*S. carolinensis*), however recent studies have shown that a poxvirus carried by grey squirrels is the likely driver of red squirrel population declines (Tompkins *et al.*, 2002).

As many wildlife species have co-evolved with the pathogens and parasites in their environment, infectious disease may be of greatest concern to wildlife populations when ecosystems are undergoing change and new elements, such as changing climate and increasing anthropogenic pressures are introduced into the mix (Thompson *et al.*, 2010b, Thompson and Conlan, 2011, Cox-Witton *et al.*, 2014). Humans may facilitate disease spread through increased urbanisation, globalised trade, increased interface between domestic and wild animals, habitat modification and destruction and other environmental changes. Anthropogenic movement of diseased animals (both domestic and wild) may contribute to disease spread, and translocation or reintroduction of wildlife can pose significant disease threats if risks are not identified and managed appropriately (Jakob-Hoff *et al.*, 2014).

Disease is of heightened concern for threatened species, where populations are small, isolated, and genetic variability may be diminished (Suzán *et al.*, 2012, Heard *et al.*, 2013). The potential for disease to act on threatened and small populations is well documented (Lafferty and Kuris, 2005, Schloegel *et al.*, 2006, Pedersen *et al.*, 2007, Wyatt *et al.*, 2008, Kriger and Hero, 2009, Gillespie *et al.*, 2015). Disease may be the ultimate driver of extinction in populations already critically diminished or under stress from other factors or disease may reduce populations to a level where other stochastic events increase risk of extirpation.

Failure to detect diseases in a timely fashion may result in magnified effects of disease in wildlife populations, as 'business as usual' management practices such as trapping, sampling and translocation may increase risk of disease spread, as has been hypothesised in the case of chytridiomycosis (Kriger and Hero, 2009). In addition, opportunities to manage or eradicate disease are missed and as disease becomes more established, the ability to influence disease dynamics is reduced.



### 1.2.1 Emerging infectious diseases

Emerging infectious diseases (EIDs) are disease-causing agents that rapidly increase in geographical range, host range or prevalence. Such events are globally recognised as occurring at an increasing rate (Daszak *et al.*, 2000). The majority of emerging infectious diseases are zoonoses (Jones *et al.*, 2008), increasing the public health significance of EIDs. The tropics are of particular interest as many EIDs originate from these areas, which also experience low levels of disease surveillance and reporting, due to logistical and socioeconomic constraints (Jones *et al.*, 2008). In investigating the possible role of disease in the decline of small terrestrial mammals in the Top End of the NT, any significant infectious disease found would most likely be considered an EID.

An emergence of disease is usually the result of interactions between multiple factors. Tompkins *et al.* (2015) list potential drivers of disease emergence in wildlife, which include:

- exposure to an infectious agent from other wild or feral populations
- exposure to an infectious agent from domestic animals (host clustering/overcrowding/stress due to habitat modification)
- host poor nutrition/food supply variation/dietary shifts
- host thermal stress/climate change
- waning host immunity/immune variation/immune system modulators
- host genetic instability/low genetic diversity/inbreeding
- favourable climate for vectors/other drivers of increasing vector numbers

These drivers are not necessarily independent and may in some cases be synergistic (e.g., waning host immunity may be caused by stress, poor nutrition or genetic issues). Many of the potential drivers listed above may apply to the environment of the Top End.

### 1.2.2 Challenges in investigating disease in free-ranging wildlife populations

Disease studies in free-ranging wildlife are often challenging and highly consumptive of time and resources. Pathogens exist in all populations, and a detailed understanding of baseline health and disease in 'healthy' populations is a necessary prerequisite to interpretation of disease information in declining wildlife populations. Baseline information is often not available, which may make interpretation of findings in declining populations challenging (Stallknecht, 2007, Ryser-Degiorgis, 2013). Wildlife disease investigations should be undertaken in a systematic, documented fashion, after appropriate assessment and prioritisation of issues has been completed. If studies are undertaken in a reactive or opportunistic manner this will significantly reduce the power of the work.

Wildlife species are by nature untamed, often cryptic in behaviour and avoid interactions with humans. Handling wildlife may present significant risk of injury to humans and in many cases, for the safety and welfare of the animal, anaesthesia must be undertaken to perform even basic examination and sampling. Sourcing and sampling sufficient wildlife cases for meaningful investigation can prove challenging. Australian native mammals are often small in size, cryptic and nocturnal, making both observational and interventionary studies difficult. In very small species, safe and ethical collection of samples, such as blood and mucosal swabs, may prove to be almost impossible as veins and mucosal sites may be too small to access.



Wildlife often occurs in areas where human densities are low and where natural vegetation levels are high, making observation and retrieval of animals (whether healthy, sick or dead) difficult. Deaths and ill-health in free-ranging wildlife, especially smaller species, are largely unmonitored and carcasses are generally lost to investigation through scavenging or decomposition. Several studies and much anecdotal evidence support the hypothesis that sick or dead animals are rarely seen in the wild (Wobeser and Wobeser, 1992, Wobeser, 2006, Wayne *et al.*, 2011). A lack of reports of diseased or dying animals does not remove disease as a potential contributor to wildlife population declines, especially in remote and well-vegetated areas. Unless wild populations are studied closely over long periods of time, the true effects of disease are easily overlooked and may be exceedingly difficult to determine.

Investigation of health and disease via post mortem examination may be one of the most definitive ways to gather information; however there are several challenges to obtaining suitable carcasses for these purposes. In wildlife studies, there are often reasonable objections to killing individuals, especially threatened species, for the purposes of disease investigation. Carcasses killed by hunters and domestic pets or as a result of motor vehicle trauma may not be representative of the population at large and are often in a poorly preserved state. Assessment of population health status on the basis of these samples may be misleading (Nusser *et al.*, 2008).

Sick or diseased wildlife may be less likely to enter traps than healthy animals, and animals in poor health may not be representatively sampled within the population. Such sampling biases are often a feature of disease investigation in wildlife, however the effects may be allowed for if the sampling biases are recognised (Stallknecht, 2007, Nusser *et al.*, 2008). In addition, diseased animals may die from primary or secondary causes (such as predation) and thus be removed from the population before sampling can occur.

By their nature, many wildlife populations are situated in remote areas, with few utilities and resources available. Equipment, expertise and commitment must be sufficient to ensure that animal welfare requirements are met at all times. Sufficient staff with expertise in recognising both healthy and diseased states in the species in question must be supported by appropriate capacity within diagnostic laboratories to test and analyse samples from non-domestic species.

Disease prevalence may vary according to season or other cyclical trends and sampling events may not be sufficiently frequent, or well distributed, to account for these variations. In the monsoonal tropics, many infectious agents are known to increase in prevalence during the wet season (e.g., arboviruses, melioidosis and *Salmonella*) whereas, for logistical reasons, most trapping and sampling is undertaken in the dry season.

In the absence of robust baseline information, experienced wildlife veterinarians and epidemiologists can make reasonable extrapolations from similar data, including those from other, closely related species or from similar geographic or ecological areas. Comparison can be made between declining populations and similar populations not undergoing decline. Additionally, studies can be actively undertaken on populations as they undergo decline. Changes in pathogen prevalence or health assessment parameters under these circumstances may provide indications that disease is playing a significant role in a decline. In many cases, where data are lacking, heuristic assumptions must be made, based on clinical expertise and 'best estimates' however such assumptions may reduce the strength of investigations. In general, wildlife disease studies will have increased power if they can be continued over a sufficient timeline to overcome cyclical variations and to allow longitudinal trends to become apparent.

Diagnostic tests for pathogens are generally developed for domestic animals or humans and have often not been validated for the wildlife species under investigation. Key factors of particular tests, such as their sensitivity and specificity, are often not known for wildlife and must be inferred as a 'best estimate' based on expert opinion.

## 1.3 Understanding disease processes

An understanding of disease and its dynamics is a necessary part of any attempts to investigate and interpret the role of disease in wildlife populations. Disease (any disturbance in the health or function of an animal) may have an infectious or non-infectious cause. Infectious diseases occur as a result of infectious disease agents (or pathogens) such as viruses, bacteria, fungi, protozoa or other parasites. Non-infectious diseases may occur associated with factors such as environmental contamination, nutritional deficiencies and genetic mutations. Development of disease involves many variables. There are several possible outcomes associated with the steps of a disease process, which play a key role in disease dynamics.

If an animal is exposed to a pathogen (e.g., virus or parasite), it may either become infected with the pathogen or no infection may occur. These outcomes are influenced by the pathogen load, the virulence of the pathogen, the susceptibility of the host species to the pathogen, and the susceptibility of the individual to the pathogen. There are many factors that influence the susceptibility of an individual to infection with a pathogen, including age, general health status, concurrent disease, previous exposure to this or similar pathogens and physiological stressors including nutrition, reproductive state, social, predatory, climatic and other environmental stressors (Wobeser, 2006).

Detailed information on the processes of infection and disease, testing for disease and interpretation of results can be found in Appendix 1.

## 1.4 General indicators of health and disease

When assessing the health and disease status of populations, it is necessary to assess the general health status of each individual examined. This allows a general assessment of population health and provides vital information to aid in interpretation of specific pathogen and disease testing. The health status of an individual may be assessed in a series of relatively simple procedures at the time of sampling, including a full physical examination (a direct assessment of the animal's body condition, weight, physical appearance and body system functionality) and an assessment of demeanour and activity levels.

Physical examinations are not able to assess health and disease at a cellular level and a range of haematological assessments and biochemical analyses can be performed to provide greater insight into an individual's physiological health and functionality. Additional indicators that may be measured as an aid to assessing health status are hormones (including cortisol and other 'stress' hormones) and the oxidative stress index, a measurement of the anti-oxidative capacity of a biological sample and an indication of the individual's potential to cope with stressors in its environment (Rabus *et al.*, 2008, Schultz *et al.*, 2011).

### 1.4.1 Macroparasites

Nearly all animals carry parasites, both internal (endoparasites) e.g., worms in the gastrointestinal tract or lungs and external (ectoparasites) e.g., ticks, mites and fleas. As with other pathogens, the presence of parasites does not necessarily equate to disease. Parasites may result in a spectrum of effects on an animal's health. At one extreme macroparasites may be considered 'normal' (non-pathogenic) and cause no perceivable ill effects in the host animal. At the other extreme they may be directly pathogenic, e.g., they will cause disease whenever they are present in that host. Many gastrointestinal parasites are considered a normal part of the animal's biosystem (Spratt *et al.*, 2008).

Ectoparasites may cause skin disease through direct effects such as parasites biting and chewing the host. More importantly they may spread pathogens between individuals and species, by feeding on blood of an infected animal and then spreading the disease agent to another individual. For example, in the Hawaiian Islands, exotic birds introduced to the area brought with them the blood borne agent of avian malaria, which is transmitted by biting insects. This novel disease caused major declines and possibly extinctions in the native bird fauna (van Riper III *et al.*, 1986, LaPointe *et al.*, 2012).

The role of parasites in animal health is complex. Poor health may be associated with a high host burden of parasites however parasite burden data needs to be carefully interpreted, as not all animals with a large parasite burden are diseased or unhealthy. The importance of a parasite may change between different hosts and may depend on the health of the individual.

## 1.5 Disease in small mammal species in Australia (with a focus on the tropical north)

Compared to other continents, there is relatively little knowledge of infectious disease in Australian wildlife, although there has been a significant expansion in this area over the past two decades. The limited information on Australian wildlife is reflective of the cryptic nature of many of our species, the lack of resources invested in this area and the relative paucity of diseases in Australian wildlife with an impact on domestic animal and human health.

In particular, there is little published literature on health investigations in small native mammal populations in Australia's north and sparse information on the potential population level impacts of disease in mammalian wildlife. Most relevant information comes from work undertaken in northern Qld, with relatively little information available from the Top End of the NT. Some information is available from studies on the same, or similar, species in other areas of Australia.

Within Australia, the population level impacts of disease in mammalian wildlife have received increasing focus in recent years. Tasmanian devil populations have been devastated by a transmissible cancer with a 100% mortality rate (McCallum, 2008). The brush-tailed bettong (*Bettongia penicillata*) has experienced significant population declines in south-west WA and it is hypothesized that infectious disease, associated with increased predation by feral cats and foxes may be driving the declines (Smith *et al.*, 2008a, Botero *et al.*, 2013, Wayne *et al.*, 2013). More details are emerging of the population level effects in koalas of chlamydial (Blanshard and Bodley, 2008, Polkinghorne *et al.*, 2013, Patterson *et al.*, 2015) and retroviral infections (Tarlinton *et al.*, 2005).

Until now, there have been no substantial studies considering the disease status in populations of native mammals in northern Australia. Little was known of baseline health and disease, nor the potential contributory role of disease in the small mammal population declines. Previous studies on health and disease in the Top End of the NT were conducted at one site (Kakadu National Park), on only two species (northern quolls, *Dasyurus hallucatus* and black rats), and on a limited number of diseases. Studies by Oakwood on northern quolls investigated two potential causes of disease: toxoplasmosis and parasite loads (Oakwood and Pritchard, 1999, Oakwood and Spratt, 2000). The study conducted by Jackson *et al.* (2010) of diseases associated with the black rat investigated six prioritised diseases (encephalomyocarditis virus; hantavirus; leptospirosis; trypanosomes; Australian leishmaniasis and angiostrongylosis) but found no evidence to support their presence in black rats in the Top End.

Woinarski *et al.* (2011) considered the potential role of disease in northern Australia mammal declines and concluded that there was little evidence at that time to support the hypothesis that disease was a significant contributor to declines. This reflected the paucity of information, at that time, on health and disease in declining populations in Australia's north and emphasized the need for focused disease studies, as the impacts of disease on wildlife (in keeping with other ecological threats to wildlife populations) are difficult to determine and may only be revealed through long-term, focused research.

A wide range of pathogens are known to infect and cause disease in mammalian species. The population declines in the Top End have affected a wide variety of mammals, across at least four different taxonomic groupings, including both marsupials and eutherian mammals (Ziembicki *et al.*, 2015). There are few currently known pathogens that can affect such a broad taxonomic range (several families and two orders) sufficiently to cause widespread population declines. No known agent is both sufficiently generalist (affecting a broad taxonomic host range), yet specific (as certain species and taxa are not affected e.g., macropods) (Pacioni *et al.*, 2015).

## 1.6 Aims of this project

This collaborative research project was developed to investigate the potential role of disease in declining native mammal populations in the tropical north of Australia's Northern Territory.

Project aims included:

- compilation and review of existing information on health and disease in small mammal species
- identification and prioritisation of disease hazards potentially impacting Top End mammal populations
- development of protocols and expertise to facilitate incorporation of health assessment and disease surveillance into field studies
- collection of data and samples from sufficient individuals within target taxa, at up to six survey locations, to allow comparison between geographical areas and between robust and declining populations
- testing of a range of biological samples for evidence of infectious disease
- analysis and description of data to determine if significant pathogen states currently exist in small mammal populations
- development of recommendations for management of health and disease in Top End populations and to guide future research
- identification and storage of suitable biological samples to facilitate future studies

## 2 Materials and methods

### 2.1 Selection of species and sites

The health and disease investigation focused on four native species:

- brushtail possum (*Trichosurus vulpecula*)
- northern brown bandicoot (*Isodon macrourus*)
- northern quoll (*Dasyurus hallucatus*)
- brush-tailed rabbit-rat (*Conilurus penicillatus*)

These species were representative of each of the major taxonomic groups believed to be undergoing decline in the Top End: Phalangeridae (possums); Peramelidae (bandicoots); Dasyuridae (carnivorous marsupials) and Muridae (rodents). We chose common species, likely to be trapped at more than one site and in reasonable numbers (to allow for suitable sample sizes), and species of relatively large body size thereby allowing for most effective sampling, including blood collection. Due to difficulties in collecting blood samples from brush-tailed rabbit-rats and their absence from some study sites, black-footed tree rats (*Mesembriomys gouldii*) were also sampled, to provide more information on health and disease in native rodents. Other native species trapped were examined and sampled opportunistically, including black rats and feral cats (*Felis catus*), which may act as reservoirs for pathogens.

Sampling focused on five key sites: Kakadu National Park, Cobourg Peninsula, Bathurst Island, Groote Eylandt and peri-urban Darwin. This allowed comparison between sites, with some sites continuing to have high trap rates and others very low trap rates. The remote nature of some sites limited the ability to perform those tests that were reliant on fresh samples being delivered to the laboratory in Darwin.

### 2.2 Development of protocols

Prior to the commencement of field work, a detailed set of written protocols were developed, describing each step of the field processes, including examination and sampling. Protocols were labelled with date and version number to allow us to keep the most recent version on file. A full list of protocols, with the most recent version number is listed in Appendix 2. All protocols developed as part of this project are available from Conservation Medicine Program, Murdoch University; email [conservationmedicine@murdoch.edu.au](mailto:conservationmedicine@murdoch.edu.au).

### 2.3 Review of information on disease in target species and in the Top End

Published and 'grey' literature and subject matter experts were consulted to collate established knowledge and expert opinion on the topic of infectious disease in small terrestrial mammal species in Australia, and in particular in the tropical north of the country.

A review of Wildlife Health Australia's ([www.wildlifehealthaustralia.com.au](http://www.wildlifehealthaustralia.com.au)) electronic wildlife health information system (eWHIS) was undertaken for the taxonomic groups Phalangeridae, Peramelidae, Dasyuridae and Muridae within the states of WA, NT and Qld from Jan 2003 to Dec 2014, with data largely drawn from free-ranging animals. A similar review of the Australian Registry of Wildlife Health's (<http://arwh.org>) electronic pathology data base was undertaken for the same four families within all areas of Australia from 1998 to 2014, with data largely drawn from captive animals, and a small number of cases from free-ranging wildlife.

A literature review was undertaken for each prioritised pathogen, once it was identified for investigation.

## 2.4 Hazard identification and assessment

In order to provide structure and transparency to the process of selecting pathogens for investigation, a hazard identification and prioritisation process, as recommended by Jakob-Hoff *et al.* (2014) and Pacioni *et al.* (2015), was undertaken before field studies commenced. This helped to guide the investigation of pathogens potentially causing disease in target species.

The collated information on infectious disease in Australian terrestrial mammals was used to develop an initial 'long list' of hazards, including all infectious agents known to have the potential to impact one or more of the target species. We acknowledge that this evidence-based process is limited by the available baseline information on health and disease in the species and geographic areas under question.

The first stage of refinement of the 'long list' was to remove all pathogens considered unsuitable for further assessment. Pathogens (or hazards) were retained on a 'short list' for further assessment if they were known, or thought likely, to cause disease in free-ranging terrestrial mammal species in Australia.

### 2.4.1 Hazard scoring and prioritisation

Information on remaining hazards was collected against the following criteria, and arranged in a matrix:

- Likelihood (L): the likelihood of the pathogen occurring in one or more of the target species
- Impact (I): the predicted population level impact of the pathogen
- Host range (R): was the pathogen known to affect a broad taxonomic range of host species?
- Geography (G): was the pathogen known, or likely, to occur in northern Australia, and in particular in the tropical north of the NT?

The 'short list', along with the information matrix was circulated (for further comment and refinement) to several veterinarians with expertise in wildlife disease, including those based in the Top End. Short-listed hazards were then scored against the above criteria, so that a quantitative system could assist in prioritisation of hazards for investigation. Likelihood was scored 1 (low) to 4 (high); Impact scored 1 (low) to 5 (significant); Host range scored 1 (narrow) to 3 (broad) and Geography scored 1 (not likely) to 3 (highly likely or confirmed). Assessing and scoring hazards in this manner ensured the strongest

focus of the ensuing investigation would be on pathogens with a broad host range and those infections likely to have a significant population level effect. Additionally, unidentified hazards and ancillary investigations (those likely to significantly increase understanding of health and disease status in target populations) were included in the assessment and scoring matrix, to help determine their usefulness to the investigation.

The formula used to develop a cumulative hazard assessment score for each hazard was:

$$\text{Total Hazard Assessment Score (HAS)} = (L \times I \times R) + G$$

As an example, *Toxoplasma gondii* was scored as follows, with a HAS of 39:

Criteria		Score
L		4
I		3
R		3
G		3
HAS	$= (4 \times 3 \times 3) + 3$	39

Three of the authors (AR, BJ and KW) independently scored each short-listed hazard. There was a high degree of consistency between each researcher in their individual scores of each hazard. Following scoring, hazards and ancillary investigations were grouped into three Priority Categories (A, B and C) according to their HAS. This scoring facilitated decisions regarding prioritisation of investigation of hazards and utilisation of ancillary testing during field and laboratory studies; see Results.

## 2.5 Field processes

### 2.5.1 Trapping

We live-trapped small mammals from July 2013 to December 2014 in six areas of the Top End (Fig. 1). Animals were trapped during routine DLRM fauna surveys or surveys led by university researchers, with DLRM or university staff leading and coordinating the field surveys. Over the course of the project three Murdoch University wildlife veterinarians and a range of other scientists and veterinary nurses participated in field trips. Additional assistance was provided by local veterinarians volunteering their time to assist with procedures.



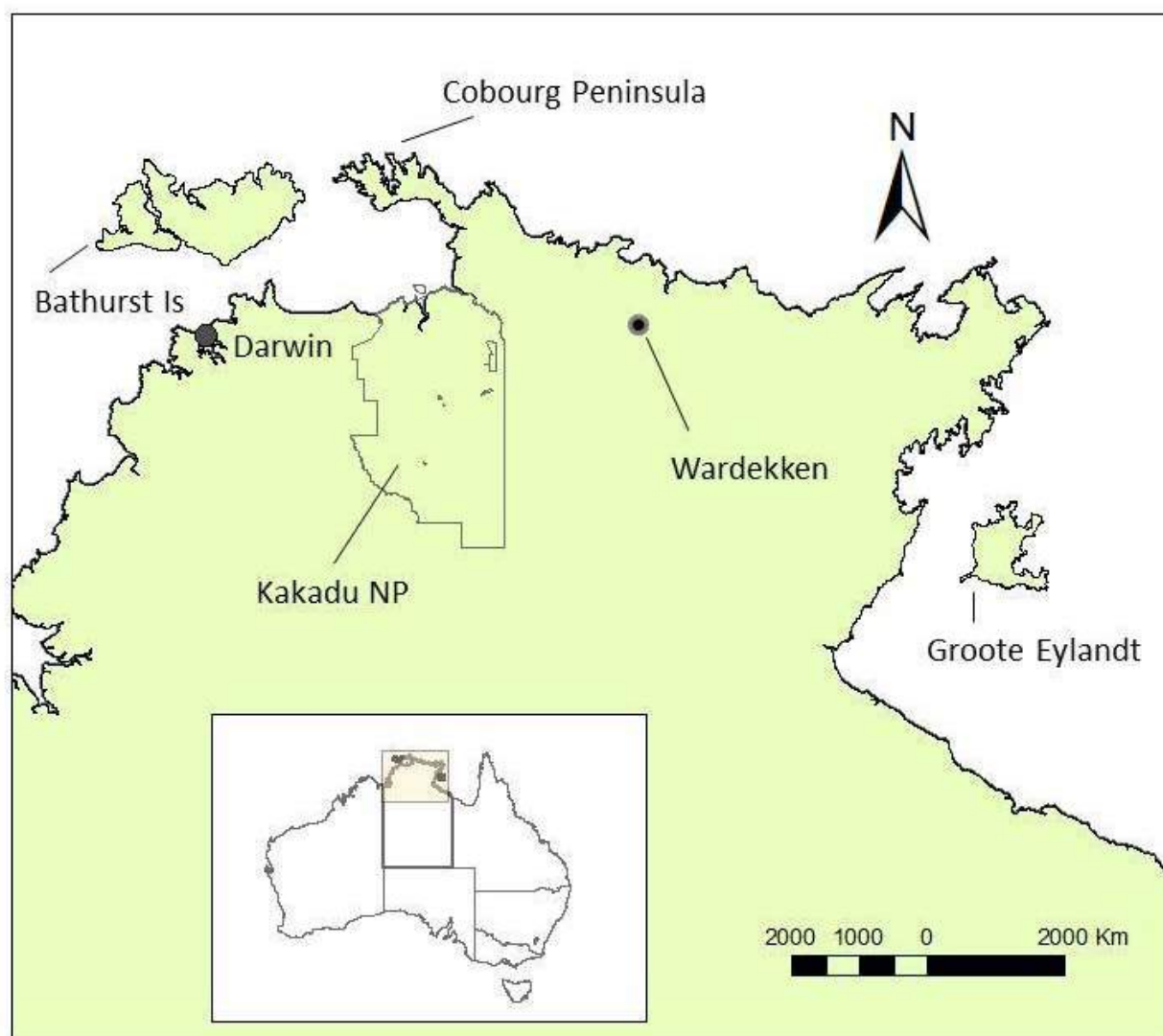


Figure 1: Map of Top End of Northern Territory showing survey areas and relationship of the Top End to the Australian continent (insert).

Traps were laid in a variety of systems but most commonly in a grid pattern with a mixture of Elliot and small cage traps. In some surveys pitfall traps were also included in trap lines. A typical trapping site consisted of 16 Elliott and 8 cage traps laid in a 50 m by 50 m square. Traps were left on site for 3-5 nights then cleaned and moved to a new site.

Traps were opened at dusk and checked within 2 hr of sunrise. If an animal was present in a trap, it was assessed for suitability for disease investigation (based on species, gender and age class). We selectively chose animals from the target species, and chose individuals to balance age class and gender distributions, where possible. Animals were held in a soft calico bag in a quiet location until processed and prior to release, and observed regularly during these times. On most trips, a maximum of eight animals a day was selected for anaesthesia and sampling. Animals not investigated were released in the vicinity of the trap, after species identification was performed under manual restraint. Additional non-invasive samples (ectoparasites and faecal samples) were collected by DLRM staff during other routine fauna surveys, when no other disease sampling was undertaken. These samples were used in some instances to expand sample sizes.



### 2.5.2 Anaesthesia

All animals undergoing full examination and sampling were anaesthetised using a portable inhalational anaesthetic machine with a precision vaporizer ('The Stinger', Advanced Anaesthetic Specialists, Gladesville, NSW, Australia) delivering Isoflurane (Delvet Isoflurane, Delvet Pty Ltd, Seven Hills, NSW, Australia) in 100% medical oxygen. Animals trapped in Darwin were anaesthetised at the Flora and Fauna Unit of DLRM. Animals trapped in remote areas were anaesthetised at a field laboratory, which varied in facility according to location. Some sites had mains power, air conditioning and fully enclosed rooms, while other studies were conducted with only generator or battery power and temporary shelter (Fig. 2).

All animals were induced and maintained using a suitable sized face-mask and a non-rebreathing circuit. During anaesthetic induction, animals were manually restrained in calico bags with the face exposed (keeping eyes covered where possible) and then removed from calico bags for full examination once a suitable level of anaesthesia was achieved.

During anaesthesia (which lasted an average 17 minutes [range 7-46 mins] from commencement of oxygen to removal of isoflurane), oxygen flow rate, isoflurane concentration, heart rate, respiratory rate, peripheral oxygenation (sPO<sub>2</sub>) and rectal temperature were recorded manually and with the assistance of a portable pulse oximeter (Nellcor, Covidien), approximately every 10 minutes. Anaesthetic data collected included the time at:

- commencement of administering oxygen
- commencement of administering isoflurane
- cessation of isoflurane
- cessation of oxygen
- recovery



Figure 2: Image of the field station at Bathurst Island in 2013.

Anaesthetic data has not been analysed but may be used in future to review anaesthetic protocols for target species.

Black rats were euthanased under anaesthesia, after sampling was completed, with an intra-cardiac injection of pentobarbitone. Feral cats were also euthanased by intravenous injection of pentobarbitone under anaesthesia, unless they were part of mark-recapture or tracking studies, in which case they were released, as for native species.

### 2.5.3 Morphometric and demographic measurements and health assessments

All anaesthetised animals received a complete physical examination whilst under anaesthesia and a range of standard data, measurements and assessments were recorded. Wherever possible we used documented, coded and numerical scoring systems to simplify processes, maximise consistency of data recording between observers and facilitate efficient analysis of data. A copy of the field data sheet can be found in Appendix 3.

The following information was collected:

- survey location, trap point and GPS coordinates
- time trap checked; time animal released; whether the individual appeared normal at release
- reproductive data: whether reproductively immature or mature and if mature, whether reproductively active or inactive
- individual's head length, foot length and head width (and scrotal width in males)
- state of the pouch (for female marsupials)
  - non parous - no evidence of previous reproductive activity in pouch
  - parous - evidence of previous reproductive activity in pouch, but no current reproductive activity
  - pouch young (PY) present
  - suckling but no PY present
  - elongated teat (indicating recently weaned young)
  - young at foot – visually confirmed
- gender and number of PY and a basic morphometric measurement (head length for larger PY, crown-rump length for small PY), where possible

If large PY were present, the pouch was taped closed with adhesive tape to minimise chance of loss of PY during handling.

Body weight was determined to the nearest 5 g using a spring balance (Pesola AG, Switzerland). Body condition was assessed subjectively based on palpation of the individual's muscle mass and superficial fat stores along the dorsum and around the tail base, and was scored on a five point scale (1=emaciation, 2=poor condition, 3=fair condition, 4=good condition, 5=obesity). Individuals with a body condition score of 2.5 or above were judged to be in 'normal' condition for their free-ranging state, based on combined clinical experience and expert opinion.

Age class was assessed subjectively as adult, sub-adult or juvenile, based on the individual's body size, morphometric measurements and evidence or otherwise of reproductive activity. Tooth wear may be used as an age indicator, despite variations in the rate of wear between individuals both within and between populations (Ryel *et al.*, 1961, Grau *et al.*, 1970, Winter, 1980, Oakwood, 2000) and may provide useful information, otherwise lacking, on age stratification in populations (Fig. 3). Molar tooth wear was assessed subjectively in all species other than rodents, and classified on a 4 point scale (nil, mild, moderate or significant wear) to assist in ageing of adults.



Figure 3: Image of a brushtail possum with significant molar tooth wear.

Health condition was assessed subjectively as either normal or abnormal, based on a range of criteria including demeanour and activity; condition of the skin and pelage; assessed body condition and the presence of obvious wounds or disease. Abnormal health conditions were further classified as mild, moderate or severe and acute or chronic in nature.

An assessment was made of the individual's species-appropriate demeanour (quiet, agitated, highly agitated) and state of alertness (unresponsive, reduced response, responsive) both in the trap and at the time of induction of anaesthesia. A highly agitated state may result in changes in some blood parameters including total and differential white cell count, cortisol and creatine kinase (Clark, 2004, Ladds, 2009). Any abnormalities in expected state of alertness may provide an indication of an individual in abnormal health.

For each major body system examined (skin and coat; head; abdomen and chest; cloaca, genitalia and pouch; and tail, limbs, toes and claws) a record was made of whether it was normal or abnormal, and if abnormal, the degree of abnormality on a three point scale (mild, moderate or severe) and whether abnormalities appeared acute or chronic in nature. The skin was observed for signs of inflammation, fur loss, wounds and ectoparasites. Any physical abnormalities were noted, investigated and treated as required.

In individuals with visible signs of infection or recent traumatic injury, a single intramuscular (IM) dose of a long-acting penicillin preparation (Norocillin L.A. Injection, Norbrook Laboratories Ltd., Corby, UK) containing procaine penicillin G (15 mg/kg) and benzathine penicillin G (11.25 mg/kg) and a single subcutaneous (SC) dose (0.2 mg/kg) of a non-steroidal anti-inflammatory medication (Meloxicam, Boehringer Ingelheim, North Ryde, NSW, Australia) were administered. All anaesthetised animals, except for some cases in July 2014, received a SC bolus of sterile isotonic Hartmann's solution (Baxter, Toongabbie, Qld, Australia) at approximately 0.5 – 1% of body weight to help counter any possible impacts from dehydration.

Digital photos and short videos were taken of representative procedures and also specific items of interest. Video footage of trapping and processing procedures was recorded professionally by Michael Lawrence-Taylor of the Northern Australia Hub of NERP.

#### 2.5.4 Blood and other specimen sampling

Biological samples collected from each individual and associated tests performed are listed in Table 1. Most samples were collected because there were specific tests identified, from the hazard prioritisation process, which required such a sample. Some samples were collected opportunistically, with the intent that they could be used for future investigative work. Some biological samples could only be collected from a sub-set of individuals.

Blood was collected, under anaesthesia, from a suitable superficial vein. The maximum blood volume collected was less than 0.5% of the individual's body weight. Most commonly utilised veins for blood collection were jugular (northern quoll); femoral (northern brown bandicoot); lateral tail (brush-tail possum); medial saphenous (brush-tail possum) and ventral tail (rodents and small dasyurids). Due to the small size of the target species, notably brush-tailed rabbit-rats, it was often difficult to collect sufficient blood samples to conduct tests.

Blood was placed into ethylenediaminetetraacetic acid (EDTA) for analysis of haematological parameters and blood borne pathogens and into plain or serum separator tubes (BD Vacutainer, Becton, Dickinson and Company, Plymouth, UK) to allow harvesting of serum. Complete blood counts were only performed when samples could be delivered within 72 hr to the laboratory. A small volume of plasma was gently withdrawn and frozen from some EDTA samples collected for analysis of blood borne pathogens, to allow for future studies on anti-oxidant capacities. EDTA samples for blood borne pathogen analysis were then frozen.

Blood smears were made immediately using fresh blood (or on occasion within 3 hr of collection from EDTA blood), air-dried then fixed with methanol (Diff Quik, Lab Aids Pty. Ltd., Narrabeen, NSW, Australia), and stored at room temperature until sent to the laboratory. One or two drops of fresh blood were placed onto small sections of Whatman paper, allowed to thoroughly air dry, labelled and stored in moisture-proof plastic bags at room temperature.

Samples for detection of herpesvirus deoxyribonucleic acid (DNA) using polymerase chain reaction (PCR) were collected using aluminium-shafted swabs (Sterile Swab Applicators, Copan Italia, Bressica, Italy) from the conjunctiva, nasal, and cloacal or urogenital tract mucosa and stored, in sterile vials, at room temperature until processed.



Table 1: Biological samples collected and tests undertaken.

\*collected from a sub-set of sampled animals.

Sample	Details	Tests performed	Archived samples available?
Whole blood	EDTA vial 1	Complete blood count (CBC)	No
	EDTA vial 2	Blood borne parasites	Samples retained by Prof Peter Irwin, Murdoch University (MU)
	Serum	Serological antibody tests for a variety of pathogens	Yes, MU
	Serum	Biochemistry profile	No
	Plasma	Archived (anti-oxidant studies)	Yes, MU
	Whatman paper	Archived (variety of pathogen tests)	Yes, MU
	Blood smears	Morphology for CBC; blood borne parasite examination	Yes, MU
Faeces	Fixed in ethanol	Direct microscopy for evidence of gut parasites (both worms and protozoa)	Yes, MU
	Frozen	Molecular examination for gut protozoal parasites	Samples retained by Prof Irwin, MU
	Frozen	Archived (cortisol studies to look at stress impact)	Yes, MU
Ectoparasites	Fixed in ethanol	Taxonomic identification Examination for blood borne parasites	Samples retained by Prof Irwin, MU
Mucosal swabs (herpesvirus)	Swabs of conjunctiva, oropharynx & urogenital area	PCR for mammalian herpesviruses	Extracted samples held by Dr Joanne Devlin, University of Melbourne
Rectal swab*	Bacterial swab	<i>Salmonella</i> culture	Yes, Murdoch Uni
Viral pathogen discovery swabs*	Rectal swab, extracted	<i>De novo</i> molecular pathogen testing	Yes, full set of all extracted samples held at MU
Fur	Frozen	Archived (cortisol studies to look at stress impact)	Yes, MU
Other microbial swab*	Frozen	Archived (bacterial culture)	Yes, MU
Skin scrapings*	Skin scrapings	Direct microscopy for evidence of skin disease and parasites	Yes, MU
Skin biopsy*	Formalin fixed	Histopathological examination for skin disease	Yes, MU
	Frozen	Bacterial culture	Yes, MU
Urine*	Frozen	Archived	Yes, MU

Rectal swabs were collected using sterile bacteriologic swabs (Sterile Transport Swabs, Interpath Service Pty. Ltd., Melbourne, Australia) and submitted to Berrimah Veterinary Laboratory (BVL) for *Salmonella* culture. Fresh rectal cultures for *Salmonella* spp. were only performed when samples could be delivered within 48 hr to the laboratory. Additional rectal swabs for *Salmonella* spp. were collected and frozen from a random sub-set of individuals during all sampling trips and subsequently submitted for culture.

Viral swabs of cloacal or rectal mucosal for both ribonucleic acid (RNA) and DNA *de novo* molecular testing and metagenomic analysis were collected using sterile nylon flocked swabs (Puritan Medical Products Co, Maine, USA), placed into Universal Transport Medium (UTM; Copan Innovation, Italy), and stored at -20°C or -80°C until extraction. These swabs were only collected in 2014, from a representative sub-set of animals at each site, with a focus on northern brown bandicoots, as this species was present at all sampling sites.

A small quantity of fur (approximately 1 g), either plucked or clipped from the dorsal hip region, was collected from each individual, wrapped in aluminium foil and then bagged and frozen at -20°C for future studies on cortisol and stress. This also provided a useful method of identifying individuals that had been recently sampled, if they were re-trapped on subsequent days.

Faecal samples were collected, when available, from inside the trap or transport bag and were held, either chilled or at room temperature, until processing (within 8 hr of collection).

### 2.5.5 Ectoparasites and skin disease investigation

The individual's ectoparasite burden, including ticks, fleas, mites and lice, was subjectively assessed on a four point scale (none, low, moderate, or heavy). Representative samples of ectoparasites were collected from all individuals with evidence of skin parasites, stored in 70% ethanol and subsequently identified by experienced veterinarians, using published literature (Roberts, 1970).

Information regarding the presence and extent of rump wear in brushtail possums, as described in Hufschmid *et al.* (2010), was not recorded as a specific item during examination. However, due to the apparently large numbers of brushtail possums observed with rump wear, its presence and severity was later estimated from clinical notes and included in analysis. Rump wear was coded as either present or absent (for brushtail possums only) and if present, coded on a three point scale: 1=mild fur changes, sometimes only visible on extremities; 2=moderate fur changes on rump and other areas; 3=significant fur wear on rump, with associated hyperpigmentation, matting of fur and evidence of dermatitis.

Skin biopsies and skin scrapings were only performed on a sub-set of individuals showing signs of skin disease. In four individuals with evidence of moderate to severe dermatitis, representative sections of affected skin were collected using a 4 mm biopsy punch (Disposable Biopsy Punch, Kai Industries Co. Ltd., Seki City, Japan). Biopsy sites were closed with an absorbable suture (4-0 Coated Vicryl, Ethicon Inc., Somerville, New Jersey, USA). Tissue samples were divided and one section was placed into 10% neutral buffered formalin, with the other stored in sterile conditions at -20°C. Skin scrapings were collected, using a scalpel blade and paraffin oil, from four individuals showing mild signs of dermatitis.

Fixed tissue samples were processed in a standard fashion for histological examination, and 5 µm sections stained with hematoxylin and eosin. Histological examination of tissues for evidence of disease and pathogens was undertaken by a veterinary pathologist experienced in wildlife anatomy and disease.

### 2.5.6 Management of samples in the field

Samples were processed in the field each day (e.g., aliquoting and fixing of faeces, centrifuging blood, harvesting and freezing serum samples) after anaesthetic procedures were completed. Samples were kept chilled in either small foam ice boxes with frozen packs, standard refrigerators or car fridges. Frozen samples were maintained in a similar manner. Due to the remoteness of many of the field sites, EDTA samples were kept chilled and haematological analyses were performed up to 72 hr after collection, or as soon as samples were received at the laboratory.

Faecal samples were aliquoted into two or three parts. Faeces for microscopic endoparasite assessment were homogenised with an approximately even ratio of 70% ethanol and stored in a plastic screw-topped vial. Blood for serum collection was allowed to clot at room temperature for 2–6 hr then serum was separated by spinning samples at 3500 RPM  $\pm$  5% for 15 min using a portable centrifuge (E8 Centrifuge, LW Scientific, Georgia, USA). Serum for biochemical and serological analysis was placed into small aliquots (0.1 to 0.3 ml) which were stored at  $-20^{\circ}\text{C}$  until processed, up to 16 months after collection.

With regards to sample quality, although all steps were taken to ensure best possible practices, temperature, humidity and transport logistics in the NT were not ideal for disease work and sample management. In September 2013 the DLRM freezer (holding most of the frozen specimens from the Cobourg 2013 trip) failed and all samples thawed to room temperature over a period of several hours. All samples were refrozen as soon as this was discovered but it is possible that the quality of frozen samples collected during the Cobourg 2013 field trip may have been compromised, particularly for biochemical analytes. We do not anticipate any other significant interference with test results due to the care taken with methods of collection, storage, and transfer of samples.

### 2.5.7 Post mortem sampling

Opportunistic post mortem samples were collected from a small number of individuals (mainly roadside carcasses from vehicle trauma). Unfortunately in almost all cases the state of decomposition was too advanced for meaningful investigation to be undertaken. Additionally, frozen brain and skeletal muscle samples were collected from euthanased feral cats and black rats on behalf of a University of Sydney researcher undertaking investigation of *T. gondii* in tissues.

## 2.6 Laboratory testing of samples

Laboratory investigations were undertaken for all Category A hazards, other than cortisol studies, as determined by the hazard prioritisation process (see Results). Cortisol studies (to assess animals' stress status) were outside the scope of the project due to time, financial and logistical restraints; however appropriate samples were collected and archived for potential future research in this area. Investigation was undertaken for four of eight Category B hazards, with additional samples also collected and archived, where possible. Seven of 26 Category C hazard groups were investigated, with most restricted to those which were possible under collaborative research agreements (thereby significantly reducing costs) and those hazards which are known to have a synergistic effect with other infectious agents (e.g. gastrointestinal macroparasites).

Samples were sent to a variety of diagnostic laboratories in Australia and New Zealand (NZ) for testing (Table 2).

Table 2: Details of diagnostic tests performed and participating laboratories.

Group	Pathogen	Test type	Sample	Laboratory
<b>Viruses</b>	EMCV	Serology for antibodies	Serum	Elizabeth MacArthur Agricultural Institute, NSW
	Herpesvirus	PCR	Mucosal swabs	University of Melbourne
	Arboviruses: Ross River and Barmah Forest viruses	Serology for antibodies	Serum	Berrimah Vet Lab, NT
	Unknown virus	<i>De novo</i> molecular sequencing	Mucosal swabs	Landcare Research, NZ
<b>Bacteria</b>	<i>Leptospira</i> spp.	Serology for antibodies	Serum	Leptospirosis Reference Centre, Qld
	Enteric <i>Salmonella</i> spp.	Faecal culture	Rectal swab/ bacterial culture	Berrimah Vet Lab, NT
	<i>Coxiella burnetii</i> (Q fever)	Serology for antibodies	Serum	Mt Pleasant Laboratory, Tasmania
<b>Protozoa</b>	<i>Toxoplasma gondii</i>	Serology for antibodies	Serum	Mt Pleasant Laboratory, Tasmania
	Trypanosomes	PCR	EDTA blood	Murdoch University
		Direct microscopy	Blood smear	Murdoch University
	Piroplasms: <i>Hepatozoon</i> and <i>Babesia</i> spp.	PCR	EDTA blood	Murdoch University
		Direct microscopy	Blood smear	Murdoch University
	Gastrointestinal protozoa	PCR	Frozen faeces	Murdoch University
<b>Helminth parasites</b>	Gastrointestinal helminths	Faecal floatation, microscopy	Ethanol-fixed faeces	VetPath, Perth
<b>Ectoparasites</b>	Ticks, fleas, lice	Investigate for haemoprotozoa	Fixed ectoparasites	Murdoch University
		Taxonomic identification	Fixed ectoparasites	Murdoch University
<b>Ancillary tests</b>	Complete blood count	Haematology analysis	Fresh EDTA blood	Berrimah Vet Lab, NT
	Biochemistry profiles	Measure analytes in serum	Serum	Berrimah Vet Lab, NT
	Skin investigation	Microscopy and culture	Skin biopsy and scrape	Berrimah Vet Lab, NT



### 2.6.1 Pathogen testing

*Salmonella* culture swabs were sent, either chilled (direct from the field; within 72 hr) or after freezing and thawing to BVL. Swabs were inoculated onto Xylose Lysine Deoxycholate (XLD, Thermo Fisher Scientific, Thebarton, South Australia [SA], Australia) agar and Brilliant Green agar (BG, Thermo Fisher Scientific, Thebarton, SA, Australia), and onto enrichment broths such as Mannitol Selenite (MS, Thermo Fisher Scientific, Thebarton, SA, Australia) broth and Rappaport Vassiliadis (RV, Thermo Fisher Scientific, Thebarton, SA, Australia) broth. The XLD, BG and MS were incubated at 35°C for 24 hr in an aerobic atmosphere and RV broth at 41.5°C. The MS and RV broths were then sub-cultured onto an additional XLD agar and BG agar plates at 24 hr post-inoculation. Both XLD plates and BG plates were read at 24 hr post-inoculation.

The suspected isolates were biochemically characterised at BVL using Microbact (Oxoid, Hants, UK) and serology (Oxoid, Hants, UK). All confirmed isolates were referred to the Australian Salmonella Reference Centre, Adelaide, SA, Australia for further identification.

**Detection of herpesvirus DNA by PCR** was performed at the Faculty of Veterinary Science, University of Melbourne, Parkville, Vic, Australia. DNA was extracted from swab samples pooled from each individual as previously described (Vaz *et al.*, 2011). Extracted DNA was used as the template in a generic pan-herpes PCR using primers targeting a conserved region of the herpesvirus DNA polymerase gene, approximately 210 – 230 base pairs in length (Chmielewicz *et al.*, 2003). DNA was then extracted and tested using the same PCR from all individual swab samples (cloacal, oropharyngeal and conjunctival swabs) for any individuals that returned positive PCR results using the pooled swabs. The PCR products were purified and their DNA sequence was determined using BDT Version 3.1 Chemistry (Applied Biosystems, Carlsbad, California, USA). Sequence and phylogenetic analysis were performed using GENEious software (Biomatters Ltd., Auckland, NZ). The predicted amino acid sequence was compared with publicly available sequences in GenBank (National Center for Biotechnology Information [NCBI], Bethesda, Maryland) using the BLASTx online algorithm and were subsequently aligned with representative members from the three Herpesviridae subfamilies from a range of host species. An unrooted maximum-likelihood phylogenetic tree was generated from this sequence alignment using the Jones-Taylor-Thornton model of amino acid replacement.

**De novo molecular testing:** This project was well suited to utilisation of new investigative techniques. Given the potential for emerging infectious diseases or novel agents to be exerting effects in mammal populations, pathogen discovery work was undertaken, on a sub-set of samples, in an attempt to address these concerns.

A collaborative research relationship was established with colleagues offering these services in NZ: Daniel Tompkins from Land Care Research (LCR) and Richard Hall from the Institute of Environmental Science and Research (ESR). *Pro bono* assistance was provided by LCR for metagenomic analysis, reducing project costs. As the work required shipment of diagnostic samples outside Australia for investigation, approval was required from the NT Chief Veterinary Officer.

Samples from one target species only (northern brown bandicoot) were tested as this was the species most widely represented across study sites. Samples were collected from a sub-set of the other target species and stored, in the event that further funds or other opportunities arise for testing these samples. Swabs were collected from the rectal or cloacal mucosa, thus only organisms from those anatomical sites could be detected. To minimise costs, samples from up to 10 bandicoots at each site were pooled and analysed as one, to allow for interpretation of differences in potential pathogens across locations. Locations for this investigation were Bathurst Island, Groote Eylandt, Cobourg Peninsula, Kakadu National Park and Darwin.

Nucleic acid (DNA and RNA) was extracted using standard processes with the Roche High Pure Viral RNA Kit (Roche, Castle Hill, NSW) at BVL and two negative lab controls using sterile water. Duplicate aliquots were made of each extraction, with one full set of extracted material stored at -80°C in Australia. Extracted samples from 38 northern brown bandicoots were shipped on dry ice to New Zealand, for *de novo* molecular testing. The samples were grouped into four pools, each representing individuals from different populations, for *de novo* metagenomic analysis. Pools consisted of swabs from Darwin individuals (n=10); Bathurst Island individuals (n=10); Cobourg Peninsula individuals (n=10) and a mix of Groote Eylandt and Kakadu National Park individuals (n=8), as only a small number of northern brown bandicoots were sampled for *de novo* molecular testing at these last two locations.

### *Sequencing*

A 100 µL aliquot of the extracted samples was pooled from the eight or ten samples from each grouping. The metagenomic method follows that previously used (Hall *et al.*, 2014). In brief, 400 µL of each of the four pooled samples was then used for nucleic acid extraction using the iPrep PureLink Virus Kit (Life Technologies, Carlsbad, California, USA) and eluted into 100 µL of molecular-biology grade water. Separate workflows targeting RNA and DNA viruses respectively were performed. Metagenomic analysis for RNA viruses required the removal of DNA using DNase treatment as per the Ambion DNA-free™ kit (Life Technologies, Carlsbad, California, USA). A 8 µL aliquot of the RNA was reverse transcribed into cDNA using a first-strand cDNA synthesis kit (Life Technologies, Carlsbad, California, USA) primed by random hexamers as per the manufacturer's instructions and also including the recommended RNase H digestion. These steps were not required for metagenomic analysis targeting DNA viruses, as the raw DNA extract alone was used. Since a minimum input amount of 1 µg of DNA is required for Illumina TruSeq™ DNA Sample Prep Kit v2 to generate libraries for sequencing, multiple displacement amplification was performed on the cDNA or raw DNA to achieve 1 µg using the Whole Transcriptome Amplification Kit (Qiagen, Valencia, California, USA), as described previously (Berthet *et al.*, 2008). The ligation and amplification steps were followed as per the manufacturer's instructions except that the ligation reaction was terminated by heating to 95°C for 5 minutes, and the amplification step was performed for 2 hours followed by termination of the reaction at 65°C for 3 minutes. Water-only (RT-PCR grade, Ambion) negative controls failed to amplify any DNA. More than 1 µg of DNA was produced for each of the four samples, which were subsequently processed by Illumina TruSeq™ DNA library preparation (Illumina, San Diego, California, USA) and sequenced as paired-end 250 bp reads on an Illumina MiSeq instrument (New Zealand Genomics Limited, Massey Genome Service, Massey University, Palmerston North, NZ).

### *Sequence manipulation and assembly*

The quality of all sequence data was checked with the FASTQC v0.10.1 software package (Babraham Institute, Cambridge). Paired-end sequence reads were labelled appropriately using the PRINSEQ-LITE v0.20.4 software package (Schmieder and Edwards, 2011) and assembled into contigs (DNA segments) using MIRA v4.9.3 (Chevreux *et al.*, 1999), using the *est*, *de novo*, and *accurate* modes, and with MIRA parameters set to remove sequences if below 20 bp or containing low complexity regions. All contigs, and unassembled reads that passed inclusion criteria, were saved into one output file. Assembly was run on the New Zealand eScience Infrastructure (NeSI) High Performance Computing Facility (Centre for eResearch, University of Auckland, NZ).

### Taxonomic assignment

Contigs were aligned separately to the entire non-redundant nucleotide database at NCBI (downloaded 27 February 2015) using, in the first instance, BLASTn in the standalone version of the BLAST+ v2.2.29 software package (Camacho *et al.*, 2009), to determine similarity to known sequences in GenBank.

**Blood borne parasites:** Molecular analysis (DNA extraction, PCR, sequencing and phylogenetic analysis) was performed at Murdoch University on EDTA blood samples. Assessment of microfilaria in blood films was not included in the original project plan. Because a high number of individuals with circulating microfilaria were detected when blood smears were manually assessed for CBC by our collaborating pathologist at BVL, we included assessment of microfilaria presence in our analysis.

**Faecal protozoal parasites:** Molecular analysis of faecal samples was undertaken at Murdoch University. Genomic DNA was extracted from faecal samples which were then analysed for the presence of *Cryptosporidium* spp. and *Giardia* spp. by quantitative PCR and nested PCR.

**Encephalomyocarditis virus serology** was performed at the Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia, using the virus neutralisation test (P Kirkland personal communication, 2014).

**Ross River virus and Barmah Forest virus serology** were performed at the Department of Primary Industry and Fisheries, Berrimah Veterinary Laboratory (BVL), Berrimah, NT, Australia. The virus neutralisation test (VNT) was performed by a modified version of the method described by Weir (2003). The testing was carried out in 96 well plates using Minimum Essential Medium [MEM] (Sigma-Aldrich, St. Louis, USA) supplemented with 10% foetal bovine serum. Due to the small amount of serum supplied, the Ross River VNT was performed using a higher dilution starting point than normal. Briefly, 50 µL of serially diluted serum in quadruplicate wells was incubated with 50 µL of MEM containing 100 tissue culture infective doses of virus for 1 h at 37°C. Then 100 µL of MEM containing 2 x 10<sup>5</sup> BSR cells (Sato *et al.*, 1977) per ml was added and the plates read for cytopathic effect after a 5 day incubation at 37°C. The serum neutralization antibody titres were calculated using the 50% end point method first reported by Reed and Muench (1938).

**Coxiella burnetii (Q fever) serology** was performed at the Department of Primary Industries, Water and Environment, Mount Pleasant Laboratories, Launceston, Tas, Australia using the complement fixation test (Ellis and Barton, 2003).

**Leptospirosis serology** was performed at the Leptospirosis Reference Laboratory, Coopers Plains, Qld, Australia using the microscopic agglutination test for antibodies to serovars Pomona, Hardjo, Tarassovi, Grippotyphosa, Celledoni, Copenhageni, Australis, Zanoni, Robinsoni, Canicola, Kremastos, Szwajizak, Medanensis, Bulgarica, Cynopteri, Arborea, Bataviae, Dlasiman, Javanica, Panama, Shermani, and Topaz of *Leptospira* spp. as described by Goris and Hartskeerl (2013).

**Toxoplasma gondii serology** was performed at the Department of Primary Industries, Water and Environment, Mount Pleasant Laboratories, Launceston, Tas, Australia using the modified agglutination test (MAT) for immunoglobulin (Ig) G antibodies to *T. gondii* and the Toxo Screen kit (BioMerieux, France). The MAT measures IgG, which rises several days after infection in most marsupial species (Desmonts and Remington, 1980). 100 uL of serum was required per animal and in some cases a Q fever serological assay was able to be performed on the same aliquot of serum.

**Gastrointestinal helminths:** Ethanol-fixed faecal samples were sent to VetPath Laboratory (Ascot, WA, Australia) and were assessed by faecal egg count (FEC), for presence and load of endoparasite ova, using a saturated salt solution and a Whitlock Universal counting chamber. Some fixed faecal samples, not sufficient in volume for FEC, were assessed via wet microscopy for presence of protozoal pathogens and nematode ova.

## 2.6.2 Haematological and biochemical testing

Packed cell volume (PCV) and total plasma protein (TPP) readings were performed in the field within 6 hr of collecting blood, using a microcentrifuge operated from a 12 V battery (ZIPocrit, LW Scientific, Inc, Georgia, USA; 11000 RPM +/-500) and a hand-held refractometer. Samples for full haematological analyses were sent, chilled, to laboratories in Darwin (BVL) and Adelaide (Gribbles Veterinary Laboratory, Wayville SA, Australia) and analyses were performed within 72 hr of collection.

Hematologic analysis at the laboratory was performed on EDTA whole blood with initial processing on an automated haematology analyser (ABC Animal Blood Counter, ABX Horiba Diagnostics, Montpellier, France) that used a different 'smart card' for each domestic species.

Multiple blood samples from northern brown bandicoots and brushtail possums were run with three different 'smart cards' (dog, cat and pig) and produced similar white cell counts (WCC) on each occasion, thus it was assumed that all three domestic animal 'smart cards' would be suitable for automated analysis of our target species. However, to maintain consistency, the dog 'smart card' was routinely used for samples submitted to BVL from this project.

In order to avoid the inherent substantial inaccuracy of using differential white blood cell counts produced by an automated analyser, differential counts were performed manually, and by the same operator for all samples submitted to BVL.

Laboratory assessment of repeated blood counts performed on the same sample over time, on a number of target species suggested that for the purposes of TWCC, the deterioration of sample over time is variable. Although there may be circumstantial or species differences in the rate of deterioration of the sample over time, submission of samples to the lab within 48 hr of collection is optimal for consistent results.

All of the red cell parameters were performed on the machine except packed cell volume (PCV) which was determined using centrifugation and microhaematocrit tubes. Total plasma protein (TPP) was determined using refractometry.

A small number of EDTA samples were processed at a different laboratory (Gribbles) using a CELL-DYN 3700 analyser (Abbott, Macquarie Park, Australia) as the facilities at BVL were temporarily unavailable. Manual differential counts for these samples were undertaken at BVL and presence of microfilaria was noted.

Haemoglobin, PCV, TPP, total red cell count (TRCC), red blood distribution, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin content (MCHC), platelet count, neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils, and basophils were measured.

Automated red cell indices (TRCC, MCV, and MCHC) are likely to be influenced by red blood cell size of the specific species to which the machine is calibrated (in our case, canine) and it is likely that they are not an accurate measurement for other species. Automated platelet counts were not performed for the majority of samples, for the same reason, but abundance of platelets was manually estimated.

Machine values for red cell indices were reported by the laboratory at our request, however these indices may be susceptible to calibrations based on species red cell size, therefore accuracy of these automated counts is questionable.

Blood smears were stained with a modified Wright-Giemsa stain (Diff Quik, Lab Aids, Narrabeen, Australia) and 100 white cells identified and counted at 400 x magnification. To look for haemoparasites, 50 fields were examined under oil immersion (1000 x).

Serum biochemical parameters were determined on an automated analyser (Konelab 20, Thermo Electron, Victoria, Australia). The following variables were measured: sodium, potassium, chloride, urea, creatinine, glucose, total bilirubin, aspartate amino transferase (AST), alanine transaminase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), globulin (Glob), albumin (Alb), albumin/globulin ratio, calcium, phosphate, magnesium, iron and creatine kinase (CK).

In some cases, our investigation was limited by the current availability of tests either commercially or via research institutions within Australia. We chose the most appropriate test for each prioritised pathogen. This decision was based on several factors including: ability to detect the disease (test sensitivity), feasibility of collecting the required sample and cost. For most of our target diseases there were limited laboratories available in Australia (or within our geographic region) with the appropriate testing regimes and expertise to analyse samples, in particular from wildlife species. Where possible, collaborations with research institutions were developed, which allowed reduction of costs and encouraged cross-institutional knowledge sharing. Sample collection, storage and transport were based on advice from the relevant specialised institution or laboratory to optimise results.

## 2.7 Data management and statistical analyses

All data was entered into an Excel spreadsheet for further analysis in 'R' version 3.0.2 (Ihaka and Gentleman, 1996).

### 2.7.1 Descriptive analyses

Apparent and true prevalence (with 95% confidence intervals [CI] estimated using the Wilson score interval) were calculated for all disease agents tested, for the entire cohort, then by location, by species, and for species and location. True prevalence calculations are derived from the test results taking into account the sensitivity and specificity of the test in question, therefore incorporating the probability of false positives and negatives. Table 3 provides the test sensitivity (Se) and specificity (Sp) used to calculate true prevalence estimates. It is important to note that for the species in this study, no Se or Sp information is available for the tests in question, and thus extrapolation from other species (domestic animals and humans) and expert opinion was necessary to estimate these parameters.

Table 3: Test sensitivity and specificity used to calculate true prevalence estimates.

Diagnostic test	Estimated test sensitivity	Estimated test specificity
<i>Salmonella</i> culture	0.7	1.0
Herpesvirus PCR	0.7	1.0
<i>Trypanosoma</i> spp. PCR	0.9	1.0
<i>Babesia</i> spp. PCR	0.9	1.0
<i>Hepatozoon</i> spp. PCR	0.9	1.0
<i>Toxoplasma gondii</i> MAT	0.95	0.95
<i>Leptospira</i> spp. MAT	0.93	0.95
<i>Coxiella burnetii</i> CFT	0.5	0.90
Encephalomyocarditis virus VNT	0.99	0.95

Selected variables that may be indicative of general health status (body condition, weight, PCV, TPP, Alb, Glob and TWCC) were analysed independent of disease result by species and location, to examine for differences. Body condition was independent of species and therefore could be examined across all species by location, as well as within species. As there are sex and species differences for body weight, this was evaluated within species by sex. The rest of the health indicators (PCV, TPP, Alb, Glob, TWCC) were evaluated within species to allow for the likely species differences between these variables. Generalised linear models (GLM) or analysis of variance (ANOVA) were used to test the significance of differences between sites, species or sexes for continuous variables (PCV, TPP, Alb, Glob, TWCC), and chi-square for significance of differences between ordinal data (body condition).

### 2.7.2 Univariate analyses

Dependent variables were the presence or absence of disease agents tested.

Measures of association were examined for nominal data (location, species, sex, season) and ordinal data (age class, body condition, tooth wear, ectoparasite burden) using the Odds Ratio (OR) with 95% CI, and p-values using the chi-square test (all categories sample sizes >5) or Fisher's exact test (any category sample size <5). Age class was determined by the observer's classification at the time of examination. In some individuals, age classification appeared erroneous (i.e. individuals were classified as adult when body weights for species and gender appeared to indicate they were immature). All such cases were confirmed from primary data sources. No attempt was made to override observer age classifications or remove this data from analysis for this report. Age classification for these individuals will be reconsidered prior to peer-reviewed publication of these findings.

Continuous data (PCV, TPP, Alb, Glob, TWCC, body weight) were checked for normality using the Shapiro-Wilk test and grouped by disease outcome. Differences for normal data (all variables) were tested for significance using ANOVA. All measures of association and difference were analysed by location, species, and then location and species, where samples sizes permitted. As for descriptive analyses, the continuous data could only be evaluated within species (and sexes or age classes) to account for differences between these groups.



## 3 Results

### 3.1 Hazard identification and prioritisation

A wide range of hazards, including viruses, bacteria, fungi, protozoa, helminths, ectoparasites and diseases of unknown origin were identified for assessment and prioritisation (Table 4).

Table 4: Long list of pathogens considered for investigation during hazard assessment.

Taxonomic group	Sub-group	Pathogen
Viruses	Systemic viruses	Adenovirus Arboviruses: Orbivirus (Wallal, Warrego and Tammar Wallaby virus) Barmah Forest virus Ross River virus Cytomegalovirus Encephalomyocarditis virus (EMCV) Flavivirus (including West Nile virus) Herpesvirus Wobbly possum virus Unknown virus
	Localised viruses	Pox, papilloma and related viruses
Bacteria	Systemic bacteria	<i>Bartonella</i> spp. <i>Bordetella bronchiseptica</i> <i>Chlamydiales</i> <i>Chromobacterium violaceum</i> Clostridial diseases including Tetanus and Tyzzer's disease <i>Corynebacteria</i> spp. <i>Coxiella burnetii</i> (Q Fever) and other rickettsiales <i>Erysipelothrix rhusiopathiae</i> <i>Leptospira</i> spp. <i>Listeria</i> spp. Meliodosis ( <i>Burkholderia pseudomallei</i> ) <i>Mycobacteria</i> spp. <i>Orientia tsutsugamushi</i> (scrub typhus) <i>Pasteurella multocida</i> <i>Proteus</i> spp. <i>Pseudomonas</i> spp. <i>Staphylococcus</i> spp. <i>Streptobacillus moniliformis</i> <i>Streptococci</i> spp. Tularaemia ( <i>Francisella tularensis</i> ) <i>Yersinia</i> spp. Other non-specified systemic bacteria
	Gastrointestinal flora	<i>Campylobacter</i> spp. <i>Escherichia coli</i> and other <i>Escherichia</i> spp. <i>Helicobacter</i> spp. <i>Salmonella</i> spp.
Fungi	Systemic fungi	Adiaspiromycosis

		<i>Aspergillus</i> spp. <i>Cryptococcus</i> spp.
	<b>Localised fungi</b>	Ringworm
<b>Protozoa</b>	<b>Systemic protozoa</b>	<i>Babesia</i> spp. <i>Besnoitia</i> spp. <i>Blastocystis</i> spp. <i>Hepatozoon</i> spp. <i>Klossiella</i> spp. <i>Leishmania</i> spp. <i>Neoplasma caninum</i> <i>Sarcocystis</i> spp. <i>Theileria</i> spp. <i>Toxoplasma gondii</i> Trypanosomes
	<b>Gastrointestinal protozoa</b>	Coccidia, including <i>Eimeria</i> spp. <i>Cryptosporidium</i> spp. <i>Giardia</i> spp.
<b>Helminths</b>	<b>Gastrointestinal helminths</b>	Not listed separately – see ‘Significant diseases and their potential impact on small mammals in the Top End – Helminth endoparasites’
	<b>Systemic and non-gastrointestinal helminths</b>	<i>Angiostrongylus</i> spp. Filaroid nematodes Other systemic pathogenic helminths
<b>Ectoparasites</b>		Ticks Fleas Lice Mites
<b>Diseases of unknown origin</b>		Choriomeningoencephalitis of brushtail possums

Hazard assessment scoring and prioritisation identified six Category A hazards and ancillary investigations, eight in Category B and 26 in Category C (Table 5). Of these, five Category A, four Category B and seven Category C hazard groups were developed for investigation. Our hazard identification process identified only one known pathogen (*T. gondii*) recognised to have the potential to exert population level effects on a broad taxonomic range of mammals within a tropical ecosystem such as the Top End of the NT.



Table 5: Assessed disease hazards and ancillary tests.

Items selected for investigation are marked in bold and highlighted.

Hazard group	Hazard	Priority Grouping
Viruses	Adenovirus	C
	<b>Barmah Forest virus</b>	<b>C</b>
	Cytomegalovirus	C
	<b>Encephalomyocarditis virus (EMCV)</b>	<b>B</b>
	Flavivirus (including West Nile virus)	B
	<b>Herpesvirus</b>	<b>A</b>
	Orbivirus (Wallal, Warrego and Tammar wallaby virus)	C
	Pox and papillomavirus	C
	<b>Ross River virus</b>	<b>C</b>
	<b>Unknown virus</b>	<b>A</b>
	Wobbly possum virus	C
Bacteria	<i>Bartonella</i> spp.	C
	<i>Campylobacter</i> spp.	C
	<i>Chlamydia</i> spp.	B
	<b><i>Coxiella burnetii</i> (Q Fever)</b>	<b>C</b>
	<i>Escherichia coli</i> and other <i>Escherichia</i> spp.	C
	<b><i>Leptospira</i> spp.</b>	<b>B</b>
	<i>Listeria</i> spp.	C
	Melioidosis ( <i>Burkholderia pseudomallei</i> )	B
	<i>Mycobacteria</i> spp.	C
	Other non-specified systemic bacteria	C
	<b><i>Salmonella</i> spp.</b>	<b>B</b>
	Scrub typhus ( <i>Orientia tsutsugamushi</i> )	C
	Tularaemia ( <i>Francisella tularensis</i> )	C
	Tyzzler's disease ( <i>Clostridium piliforme</i> )	C
	<i>Yersinia</i> spp.	C
Fungi	<i>Aspergillus</i> spp.	C
	<i>Cryptococcus</i> spp.	C
Protozoa	<b><i>Cryptosporidium</i>, <i>Giardia</i>, <i>Blastocystis</i> and <i>Coccidia</i> spp.</b>	<b>B</b>
	<i>Leishmania</i> spp.	C
	<b>Piroplasms including <i>Babesia</i>, <i>Hepatozoon</i> and <i>Theileria</i> spp.</b>	<b>C</b>
	<b><i>Toxoplasma gondii</i></b>	<b>A</b>
	<b>Trypanosomes</b>	<b>C</b>
Macroparasites	<i>Angiostrongylus</i> spp.	C
	<b>Ectoparasites including ticks, fleas, lice and mites</b>	<b>C</b>
	<b>Gastrointestinal nematodes, trematodes and cestodes</b>	<b>C</b>
	Other internal macroparasites	C
Ancillary investigations	Anti-oxidant assessment	B
	<b>Biochemical analysis</b>	<b>A</b>
	<b>Complete blood count</b>	<b>A</b>
	Cortisol studies	A

## 3.2 Wildlife Health Australia and Australian Registry of Wildlife Health data

Wildlife Health Australia electronic data for the taxonomic groups Phalangeridae, Peramelidae, Dasyuridae and Muridae within the states of WA, NT and Qld from Jan 2003 to Dec 2014 revealed only a small number of reported cases within northern areas of Qld and WA and within the NT. Within the NT most reports were of apparently sporadic disease in individuals, including several cases of dermatitis in various species and lesions consistent with stress-related ulcerative dermatitis in two brushtail possums, as described in this species in other areas of Australia (Johnson and Hemsley, 2008).

There were no recorded disease events from the far north of WA. Of disease events reported elsewhere in WA, most notable were several reported cases and clusters of toxoplasmosis (confirmed by histopathology) in southern brown bandicoot (*Isodon obesulus*). There were also reports of *Chlamydia* spp. in populations of western barred bandicoot (*Perameles bougainville*) and viral induced papilloma-like lesions in southern brown bandicoots. Within Qld there were multiple reports of toxoplasmosis and other neurological conditions in brushtail possums with few reports in other taxa.

Findings of relevance to our study from a review of the Australian Registry of Wildlife Health data are summarised below:

**Dasyuridae:** Mycobacterial disease, cryptococcosis, sarcocystosis, cataracts, liver fluke, microfilaria, abdominal tapeworm, neoplasia and demodex-like mange were reported.

**Peramelidae:** Mycobacterial disease, cryptococcosis, sarcocystosis, unclassified protozoal disease, pneumonia, neoplasia and trauma were reported.

**Muridae:** Cataracts, cryptococcosis, salmonellosis, neoplasia and a wide range of ectoparasites and endoparasites were reported.

**Phalangeridae:** Mycobacterial disease, coccidiosis, dermatitis, sarcocystosis, pneumonia, microfilaria, encephalitis, neoplasia and trauma were reported.

## 3.3 Existing knowledge of disease in target taxa

The following section briefly reviews current knowledge of disease in the four target species of this study, in the wider Australian context: brushtail possum, northern brown bandicoot, northern quoll and brush-tailed rabbit-rat. Where relevant, reference is made to reports of disease from animals within the same family or genus as the target species. Much of the information below has been summarised from Vogelnest and Woods (2008) and Ladds (2009), unless indicated otherwise.

In many scientific reports there is a description of an infectious agent in native species; however in many cases no disease has been associated with the agent. Much of the current information on native mammal health and disease is derived from a captive or rehabilitation context and may not be relevant to free-ranging wildlife. Increased and unique reports of disease events in captive animals may be in part due to observational opportunities but may also reflect issues of stress and possible immunocompromise associated with captivity. It is important to take care when extrapolating knowledge of disease in captive wildlife to free-ranging populations (Ladds, 2009). Limited studies in the Top End have involved occasional cases submitted for pathology by private veterinary practitioners, wildlife carers or fauna parks; necropsies of carcasses retrieved from motor vehicle trauma and euthanized trapped animals (C Shilton, personal communication 2014).

### 3.3.1 Dasyurids and northern quolls

There is a relatively large amount of health and disease information on Tasmanian devils and scattered information on other species of dasyurids. Holz (2008) reviewed current information. Most references refer to species other than northern quolls.

A three year study of northern quolls in Kakadu National Park looked at factors contributing to population decline, including a limited assessment of potential disease factors and concluded there was no evidence of *Toxoplasma gondii* infection (Oakwood and Pritchard, 1999). An assessment of ecto- and endoparasites found a range of parasites, including ticks, a trombiculid mite, a louse, fleas, and 17 species of endoparasite including a trematode, a cestode, nematodes, a pentastome and a protozoan (*Sarcocystis* sp.). Most individuals had low parasite burdens and it was concluded that parasitism was not a major contributing factor to declines of northern quolls at that time (Oakwood and Spratt, 2000).

Diseases primarily seen in captive dasyurids (and almost certainly associated with captive husbandry factors) include Tyzzer's disease; bacterial pneumonia (especially due to *Pasteurella* spp. and *Pseudomonas* spp.), mycobacterial disease, cataracts and cryptococcosis.

#### 3.3.1.1 Viruses

Inclusion bodies from cytomegalovirus have been seen in a range of small dasyurids but associated disease has not been confirmed.

#### 3.3.1.2 Bacteria

A range of serovars of *Salmonella* have frequently been isolated from dasyurids and are generally considered incidental findings, although all *Salmonella* serovars are considered capable of causing disease. Other gastrointestinal bacteria including *Helicobacter* spp. and *Campylobacter* spp. have been reported in dasyurids but with no associated signs of disease. These bacteria are considered a possible cause of gastroenteritis in any mammalian species.

Cutaneous mycobacteriosis has been seen in spotted tail quolls and Tasmanian devils as a result of infection with soil-living *Mycobacteria* spp. but is recognised sporadically only in captive animals. *Pasteurella multocida* caused fatal disease in a captive northern quoll in Darwin.

#### 3.3.1.3 Protozoa

A range of blood borne protozoa, including *Hepatozoon*, has been reported in quolls and other dasyurids, without associated disease. *Babesia* spp. has been implicated in fatal anaemia in antechinus (Clark, 2004). *Sarcocystis* has been reported in northern quolls, with no apparent association with disease (Oakwood and Spratt, 2000). Clinical disease associated with *Toxoplasma* infection is rarely reported in dasyurids.

### 3.3.1.4 Helminths

Many species of gastrointestinal helminth parasites have been reported in dasyurids including quolls. Both a liver nematode (*Sprattia capilliforme*) and a nematode in the tongue have been described in northern quolls although disease has not been associated with either parasite. Spargana (stages of worms found subcutaneously and in body cavities) are not uncommon in dasyurids and although generally not associated with disease have, on rare occasions, been associated with mortality in northern quolls (Clark, 2004). Pentastomids are reported to cause granulomas in the abdomen and liver of northern quolls and may be responsible for significant disease.

*Angiostrongylus* spp. (the rat lungworm which migrates through the brain of the host) has been reported in other dasyurids species.

### 3.3.1.5 Ectoparasites and skin disease

Demodectic mange has been seen in a range of dasyurids, and sarcoptic-like mange in larger species of dasyurids, resulting in skin disease. Six species of ticks, 32 species of fleas and lice from the genus *Boopis* have been recorded in dasyurids. Patchy fur loss due to seasonal moult is commonly seen in dasyurids including quolls. Multiple, purulent skin lesions are frequently reported in wild spotted tail quolls of unknown cause, with multifactorial factors most likely involved.

### 3.3.1.6 Other conditions

Dasyurids as a group suffer from high rates of neoplasia; the reasons for this are not known. Neoplasia is more common in aged animals and is likely only to have a significant impact in captive populations, which have extended longevity compared to free-ranging individuals of the same species. Degenerative neurological conditions and spinal disease have frequently been seen in aged, captive dasyurids, including quolls. A relatively high incidence of cataracts has been seen northern quolls in captivity and is most likely related to captive husbandry. Of note is the impact on northern quoll populations of cane toads due to toxicity from bufotoxins. Quolls as a group are considered very sensitive to this toxin.

## 3.3.2 Bandicoots and northern brown bandicoots

Bandicoots in general are a well-studied group. There is a relatively large amount of information on health and disease in this taxon however most of it derives from southern and western Australian states. Lynch (2008) reviewed current information. Unless specifically stated, information refers to species other than northern brown bandicoots.

Diseases primarily seen in captive animals (and almost certainly associated with captive husbandry factors) include periodontal disease, bacterial pneumonia, mycobacterial disease and cryptococcosis.

### 3.3.2.1 Viruses

A novel virus with similarity to both papillomavirus and polyomavirus has been found to cause disease in western barred bandicoots and is considered a significant factor in the conservation of the species (Woolford *et al.*, 2007). Liver disease, possibly associated with a herpesvirus, has been seen in bandicoots. Acute heart disease, possibly associated with an unknown viral agent, has also been seen in bandicoots.

### 3.3.2.2 Bacteria

Mortality from generalised bacterial infection is not uncommon in bandicoots. Mycobacteriosis has been reported in captive bandicoots including northern brown bandicoots. Bacterial septicaemia (reported in captive animals) is sometimes seen as a sequel to environmental contamination of wounds. *Escherichia coli* and other bacteria including *Proteus* spp. have been reported. Wild bandicoots are often attacked by cats and frequently succumb to *Pasteurella multocida* septicaemia from infected bite wounds. Septicaemia due to *Erysipelothrix rhusiopathiae* has been reported including in northern brown bandicoots; the infection is dependent on an environmental source for the bacteria. *Chlamydiales* have been potentially implicated in ocular disease in wild western barred bandicoots (Warren *et al.*, 2005).

*Orientia tsutsugamushi* (the cause of scrub typhus and a rickettsial organism related to *Coxiella burnetii*) and has been reported in bandicoots in north Qld.

### 3.3.2.3 Protozoa

The coccidian protozoa (*Eimeria* spp.) very commonly infect the gastrointestinal tract (GIT) of bandicoots and are rarely associated with disease. Occasionally GIT signs are seen in young or stressed animals in captivity. Disseminated infections with coccidia-like organisms resulting in death have been reported in captive bandicoots. Infection of kidney tissue with *Klossiella* spp. is a common finding in bandicoots and is not associated with disease. *Sarcocystis* spp. have been seen in bandicoots with associated localised pathology.

The blood borne parasites *Babesia* spp., *Theileria* spp. and *Hepatozoon* spp. are all reported in bandicoots with no disease associated with these protozoa. Trypanosomes have been reported in several species of bandicoots, including the northern brown, but with no associated disease. Bandicoots are considered susceptible to infection with *Giardia duodenalis* but its significance in wild bandicoot populations is not known. This parasite has been implicated in the death of a captive juvenile northern brown bandicoot.

Bandicoots in general are considered highly susceptible to toxoplasmosis. Infection may result directly in death or cause a range of clinical signs that may predispose to other events such as predation. Recrudescence of toxoplasmosis is commonly reported in bandicoots following immunosuppressive events.

### 3.3.2.4 Helminths

Lungworms have been reported in bandicoots but are not normally associated with disease; they have been reported to cause lung abscesses in northern brown bandicoots (Norman, 1991).

*Physaloptera perameles* and related species of stomach worm may cause disease in most bandicoot species if parasite loads are sufficiently high. *Gnathostoma* spp. is reported to cause stomach pathology in northern brown bandicoots (Norman, 1991). *Capillaria* spp. have been reported in various organs and muscles of bandicoots but are not associated with disease. Other strongyles of the gastrointestinal tract may occasionally cause disease (notably in captive juveniles) but most infections are not clinically significant. Heavy burdens of *Echinoderma* spp. may be seen in the small intestine of northern brown bandicoots (Norman, 1991). Larval stages of nematodes have also been reported subcutaneously and within body cavities and may cause disease. *Angiostrongylus* spp. have not been reported in this family other than in the bilby, but could potentially cause disease.

### 3.3.2.5 Ectoparasites and skin disease

Heavy ectoparasite burdens are seen occasionally in wild bandicoots; high levels of ticks may contribute to poor health. At least three species of ticks have been reported in northern brown bandicoots: *Haemaphysalis humerosa*, *Ixodes tasmani* and *I. holocyclus*. Heavy burdens of paralysis ticks may be detrimental to juvenile northern brown and other bandicoots. The mites *Haemolaelaps marsupialis* and *Odontacarus* spp. and occasional sarcoptic-like mange have been reported in bandicoots.

### 3.3.2.6 Other conditions

Acutely fatal kidney disease, possibly due to toxins, has been reported in captive eastern barred bandicoots from a number of different facilities. Ocular diseases of unknown origin and neoplasia have also been reported in captive bandicoots. Trauma (including from introduced predators and vehicles) has been a major cause of mortality in managed programs for other free-ranging bandicoot species. Bandicoots are considered susceptible to environmental toxins such as organochlorines, which persist in the environment and may be bio-accumulated by bandicoots, due to their feeding ecology.

## 3.3.3 Phalangeridae and brushtail possums

There is a relatively large amount of information on disease in possum species, in particular the brushtail possum (this species is both widespread and common and is also a major introduced pest in NZ). There is little available information on the health and disease of brushtail possums in the Top End. Johnson and Hemsley (2008) reviewed current information on possums.

Diseases primarily seen in captive animals (and almost certainly associated with captive husbandry factors) include *Yersinia* spp. infection, *E. coli* infections, Tyzzer's disease, gastrointestinal bloat, bacterial pneumonia, *Candida* spp. infections of the GIT, cataracts, metabolic bone disease, ringworm and cryptococcosis.

### 3.3.3.1 Viruses

Many viruses have been investigated in brushtail possums in NZ, in part for their potential as biological control agents. Many of these pathogens have not been reported in brushtail possums in Australia.

Serological evidence for Ross River virus and Murray Valley encephalitis has been reported in brushtail possums without associated signs of disease. A probable herpesvirus infection and mild clinical disease associated with papillomavirus and poxvirus have been reported in brushtail possums.

Wobbly possum syndrome (WPS) has been extensively reported in brushtail possums in NZ. This was recently confirmed to be caused by a nidovirus (Dunowska *et al.*, 2012). This virus is not known to occur in Australia; possums in Australia presenting with similar neurological disease are thought to be suffering from a different syndrome. Choriomeningoencephalitis (inflammation of central nervous system [CNS] and eyes) of unknown but presumed viral origin has been seen in wild brushtail possums in southeastern Australia. Signs are similar to WPS; individuals are docile, uncoordinated, confused, lose fear of predators, lose body condition and appear blind (Ladds, 2009).

### 3.3.3.2 Bacteria

Wild brushtail possums are reported to carry *Salmonella* spp. at a high prevalence and a range of serovars have been detected. In most cases *Salmonella* is not considered a significant cause of disease, with occasional exceptions in captive situations.

Leptospiral infection is commonly reported in brushtail possums in NZ (up to 80% of adults) where brushtail possums are considered maintenance hosts of one of the serovars. Lower levels of infection are generally seen in brushtail possums in Australia. Infection may cause kidney disease in brushtail possums, but in general, disease in this species is considered mild.

*Chlamydiales* have been reported in mountain brushtail possums but are not necessarily associated with disease. Serological evidence of exposure to *C. burnetii* and *Rickettsia australis* (the cause of Queensland tick typhus) have been detected in brushtail possums, but with no associated signs of disease.

*Mycobacteria bovis* (bovine tuberculosis) has only been reported in brushtail possums in NZ (this disease has been eradicated in Australia and was never seen in possums in Australia prior to this). The high prevalence in brushtail possums in NZ is believed to be due to differences in the host species ecology in NZ.

Tyzzler's disease has been reported in free-ranging brushtail possums as well as captive individuals, mainly in juveniles. The organism survives in the environment and infection is generally associated with some degree of immunosuppression.

Bacterial abscesses and infected joints in possums are most commonly associated with trauma and immunosuppression. Bacterial septicaemia and wound infections are seen after motor vehicle trauma, conspecific aggression and bites from predators, particularly cats.

### 3.3.3.3 Protozoa

Coccidian protozoa, including *Eimeria* spp., are commonly reported in the GIT of brushtail possums; infection does not usually result in disease although diarrhoea has been seen. *Klossiella* spp. have been seen in the kidney of a common ringtail possum (*Pseudocheirus peregrinus*), with no link to disease. *Sarcocystis* spp. is reported in the muscle of brushtail possums but is not usually associated with disease.

*Toxoplasma* infection is seen in brushtail possums and has been reported to cause acute mortality, including in free-ranging individuals, however compared to bandicoots, infection is more often sub-clinical. Serological evidence of *T. gondii* infection has been seen in clinically normal brushtail possums and clinical disease is often associated with immunosuppression.

### 3.3.3.4 Helminths

A range of endoparasites have been reported in possums but are rarely reported to cause disease. *Marsupostrongylus minesi* is a common lung nematode; occasionally pneumonia has been associated with this parasite, sometimes in free-ranging possums. Angiostrongylosis is reported in brushtail possums in Qld and NSW, associated with neurological signs.



### 3.3.3.5 Ectoparasites and skin disease

A range of ectoparasites have been reported in possums and heavy burdens may cause disease, particularly in young animals. Sarcoptic mange has been reported in possums. Rump wear (a syndrome of hair loss and dermatitis primarily of the rump) is seen in wild populations and is thought to be associated with environmental stressors. Swollen paw syndrome (a syndrome of unknown aetiology with possible bacterial involvement) is reported only in common ringtail possums.

### 3.3.3.6 Other conditions

Stress as a disease factor has been recognised in free-living brushtail possums; it may be associated with climatic stress, territorial competition and overcrowding and is probably one of the factors contributing to rump wear. Emaciation of wild brushtail possums may be seen secondary to lack of food resources and overcrowding. Trauma from vehicles and dogs (sometimes cats) is commonly reported in free-living brushtail possums. Bite wounds may become infected with a range of bacteria, which may result in septicaemia and death. Possums also suffer from a range of spontaneous neoplasms.

## 3.3.4 Native rodents

There is relatively little information on native rodent health and disease and most is derived from observations in captive species. Breed and Eden (2008) reviewed current information.

Diseases primarily seen in captive animals (and almost certainly associated with captive husbandry factors) include Tyzzer's disease, respiratory infections (including cilia-associated respiratory disease), chronic progressive kidney disease and cataracts of the eye.

### 3.3.4.1 Viruses

Antibodies to encephalomyocarditis virus have been found in a range of rodent species; however infection is generally thought to be asymptomatic. Cytomegalovirus and associated disease has been seen in wild rodents in Qld but the significance of this virus is not known (Ladds, 2009).

### 3.3.4.2 Bacteria

Many serovars of *Salmonella* have been reported in Australian rodents although associated disease has not often been reported in free-ranging individuals.

*Leptospira* spp. have been reported from a range of Australian rodent species and in most cases it appears that infection is asymptomatic.

*Burkholderia pseudomallei* (melioidosis) has been reported to cause suppurative pneumonia in two rodent species in northern Qld. *Streptobacillus moniliformis* (the cause of rat bite fever) has been found in captive and wild rodents in Australia but does not appear to cause disease in the rodent host.

*Orientia tsutsugamushi* (scrub typhus) has been reported in a range of rodent species in northern Qld but no pathology associated with infection has been seen. Other rickettsial organisms have been reported in rodents from other areas of Australia however no associated disease has been seen.

### 3.3.4.3 Protozoa

*Toxoplasma gondii* has been reported in Australian rodents and may cause disease or inapparent infection. *Sarcocystis murotechis* has been seen in various Australian rodent species, with cysts in skeletal muscle but no associated disease. Blood borne parasites including *Trypanosoma* spp. and *Hepatozoon* spp. have been reported in rodents. Severe *Hepatozoon muris* infection may cause anaemia and splenomegaly (Clark, 2004).

### 3.3.4.4 Helminths

Few comprehensive studies of helminth parasites of Australian rodents have been undertaken however it is known that typical rodent gastrointestinal helminths consist mainly of nematodes (round worms).

*Angiostrongylus cantonensis* (the rat lungworm) has been found in black and brown rats but is not reported to infect native rodents. The lungworm *Gallegostrongylus australis* may cause mild to significant disease in some Australian rodent species. More serious disease is likely due to transmission of the parasite into previously unexposed and therefore naive hosts.

### 3.3.4.5 Ectoparasites and skin disease

A range of ectoparasites have been reported although none have been strongly associated with disease in free-ranging native rodents.

## 3.4 Significant diseases and their potential impact on small mammals in the Top End

For each pathogen selected for investigation the following section summarises: a brief description of the agent and associated disease; current knowledge of the disease in Australian mammals; and, where available, information specific to the NT or tropical north of Australia. Additional brief notes on non-prioritised pathogens are provided in Appendix 4.

### 3.4.1 Viruses

#### 3.4.1.1 Barmah Forest virus

Barmah Forest virus (BFV) is a zoonotic Alphavirus transmitted by mosquitoes and human cases are recorded each year in the Darwin region (Kurucz *et al.*, 2015). It has been thought that the main vertebrate hosts for BFV may be bats or birds, although this is not confirmed (Russell and Kay, 2004). Brushtail possums have been shown to develop antibodies to BFV but may not produce sufficient amounts of virus to infect mosquitoes (Boyd *et al.*, 2001).

Previous serological investigations show that dogs, cats, horses, flying foxes, quokkas and brushtail possums in other states of Australia may carry antibodies to BFV (Johansen *et al.*, 2005, Kay *et al.*, 2007). It is not known how long antibody levels persist in marsupial hosts after infection. BFV infection in wildlife has not been associated with disease.

### 3.4.1.2 Encephalomyocarditis virus

Encephalomyocarditis virus (EMCV) is capable of infecting a wide range of animals including humans. Different host species vary in their susceptibility to both infection and disease. In humans and other susceptible species, infection often results in sudden death, generally due to myocarditis (inflammation of the heart muscle). Infection is not always fatal and some individuals may show no signs of disease (Thomson *et al.*, 2001). Disease associated with EMCV is generally sporadic but outbreaks have occurred in pigs (Acland and Littlejohns, 1975) and in zoo animals with deaths across a range of species including macropods (*Macropus rufogriseus*, *M. rufus* and *Dendrolagus goodfellowi* and *D. lumholtzi*) (Wells *et al.*, 1989, Reddacliff *et al.*, 1997, McLelland *et al.*, 2005, Carocci and Bakkali-Kassimi, 2012, Pacioni *et al.*, 2014). EMCV has been reported in water rats (*Hydromys chrysogaster*) in north Qld (Pope, 1959) and is suspected as the cause of death of a rock rat (*Zygomys* spp.) (Ladds, 2009). The virus has also been recovered from an opossum (*Didelphis virginiana*) (Thomson *et al.*, 2001). The potential impact of the disease on Australian native mammals is largely unknown.

EMCV is a very resistant virus and may remain infectious for days, even in a hostile environment, and for up to 18 m under optimal circumstances (Carocci and Bakkali-Kassimi, 2012). Although the role of rodents in the epidemiology of EMCV has not been clarified, they are generally considered the reservoir species for this virus. A potential role for wild pigs as a reservoir species has also been identified (Carocci and Bakkali-Kassimi, 2012). It is likely that infection in other animals is a result of ingestion of either infected rats or rat faeces (during foraging behaviour). In black rats, the assumed carrier species, infected individuals generally exhibit no signs of clinical disease.

It is not known how long naturally acquired antibody levels persist in marsupial hosts after infection, however vaccine-induced antibodies persisted for at least 6 months in wallabies and other mammals (McLelland *et al.*, 2005).

### 3.4.1.3 Herpesvirus

Herpesviruses (HV) infect a range of wide range of mammalian species. Herpesviruses may vary in their host range and pathogenicity (Roizman and Pellett, 2001, Smith *et al.*, 2008b, Vaz *et al.*, 2011, Wilcox *et al.*, 2011, Stalder, 2013, Amery-Gale *et al.*, 2014). Most HV are capable of forming latent (long-lasting infections) with intermittent shedding and some are linked to the development of cancer. HV in some mammalian hosts have been associated with significant mortality, particularly in naive hosts. Many HV infections are benign, resulting in little or no clinical signs. The expression of HV-related disease is often associated with stressors in the host's environment and immune-compromise (Ladds, 2009). The virus is highly infectious but does not survive for extended periods in the environment and is generally transmitted during close contact. As such, population density and contact rates will play a role in the epidemiology of the disease (Castro, 2001, Stalder, 2013).

Clinical signs associated with HV range from lesions on mucosal surfaces, respiratory signs, reduced fertility, development of cancerous lesions, hepatitis, severe systemic disease and sudden death (Ladds, 2009). Illness associated with HV is most commonly reported in captive animals, which is likely due to observational opportunities but may also reflect stressors related to captivity. There is currently limited knowledge of the significance of HV infection in Australian marsupials (WHA, 2013a). Most knowledge of HV affecting Australian native mammals is in macropods, however recent studies have investigated HV in a range of other species (Vaz *et al.*, 2011, Stalder, 2013, Amery-Gale *et al.*, 2014). HV has been reported to cause disease in black rats in Australia and there is a possibility that infection may be transmitted to native rodents (Ladds, 2009).

### 3.4.1.4 Ross River virus

Ross River fever is caused by an Alphavirus transmitted by mosquitoes, Ross River virus (RRV). It is an important zoonotic disease of humans in Australia and RRV cases are recorded each year in humans in the Darwin region (Kurucz *et al.*, 2015). Macropods have traditionally been seen as a significant reservoir host for RRV however it is known that a very wide range of vertebrates may carry the virus (Russell and Kay, 2004). Previous serological investigations of dogs, cats, horses, flying foxes and brushtail possums in Brisbane suggested these species may be involved in RRV transmission (Kay *et al.*, 2007). Macropods are relatively uncommon in peri-urban Darwin indicating there may be mammals, other than wallabies, involved in urban RRV transmission cycles in Darwin (Vale *et al.*, 1991).

RRV has not been associated with clinical disease in Australian native wildlife (Harley *et al.*, 2001, Ladds, 2009). It is not known how long antibody levels persist in marsupial hosts after infection.

### 3.4.2 Bacteria

Bacterial infections causing serious, acute disease in free-ranging Australian mammals are relatively rarely reported.

#### 3.4.2.1 *Coxiella burnetii* and other rickettsiales

*Coxiella burnetii* is a rickettsial bacterium which causes Q fever (QF) and is a zoonosis. Several other related rickettsial organisms including *Orientia tsutsugamushi* (scrub typhus) and *Rickettsia australis* (Queensland tick typhus) cause zoonotic disease in Australia. However the characteristics of *C. burnetii* make it more infectious than many other rickettsial organisms. It survives extended periods of time in the environment and can cause infection with an extremely small dose (CFSPH, 2007).

Q fever is found in most regions of the world and is considered endemic in the northern states of Australia (Garner *et al.*, 1997, Schultz *et al.*, 2007). The pathogen has two infection cycles (through domestic/ feral ruminants and through marsupials, where transmission occurs via ticks). A variety of ticks have been found to naturally transmit the pathogen to various wildlife species. There is limited information on infection and the potential for disease in wildlife and on the role of native mammals as reservoirs for *C. burnetii*. Bandicoots, macropods and rodents are considered likely reservoirs for *C. burnetii* in Australia (Derrick and Smith, 1940, Garner *et al.*, 1997, WHA, 2013b).

Serological surveys have indicated that *C. burnetii* and other rickettsiales occur in a wide range of Australian marsupials and rodents, however in most cases clinical disease is considered mild or absent (Cooper *et al.*, 2012, Cooper *et al.*, 2013). *C. burnetii* is known to cause serious disease in a wide range of mammal species, including humans (CFSPH, 2007, WHA, 2013b) and the potential exists for it to be associated with disease in Australian mammals. An experimental infection of a rufous bettong (*Aepyprymnus rufescens*) with *C. burnetii* resulted in fatal disease, however northern brown bandicoots similarly infected did not develop clinical disease (Derrick *et al.*, 1940).

The incidence of disease in humans in the NT appears to be increasing (Schultz *et al.*, 2007). In many cases in the NT, a 'traditional' route for infection (i.e. exposure to the pathogen through domestic livestock) has not been found, raising the speculation that humans may be acquiring *C. burnetii* via other routes, including exposure to wildlife (C Shilton personal communication, 2014).

It is not known how long antibody levels persist in wildlife hosts after infection, however it is known that hosts can remain persistently infected for prolonged periods of time and a detectable level of antibodies may indicate either a previously infected animal or a persistent carrier of the disease (CFSPH, 2007, WHA, 2013b).

### 3.4.2.2 *Leptospira* spp.

*Leptospira* is a genus of bacteria causing leptospirosis, a disease of both humans and animals. Leptospirosis is zoonotic and is considered a significant public health risk in the NT (Centre for Disease Control, 2012). There are many types of leptospires, some non-pathogenic and some highly pathogenic (Wobeser *et al.*, 2001). Leptospirosis has been identified as a globally emerging infectious disease, having an increased incidence particularly in tropical areas of the world (Bharti *et al.*, 2003).

Leptospiral organisms reside in the kidneys of carrier animals, from maintenance host species, which almost invariably do not develop disease from infection. Infection can be transmitted to 'accidental' host species, which are susceptible to developing clinical disease. The organism needs warmth and moisture to survive in the environment and as a result there is a seasonal pattern to infection in tropical areas (Wobeser *et al.*, 2001, Centre for Disease Control, 2012). Leptospirosis is spread through contact with urine from infected animals (especially rodents, possums and cattle, as well as pigs and dogs). This may include contact with urine-contaminated soil/water/food, or through broken skin and mucous membranes. A role for sexual transmission has also been reported in some species (Hathaway *et al.*, 1978). In humans, signs of infection range from asymptomatic to severe (occasionally fatal) disease from kidney damage, meningitis or liver failure (Centre for Disease Control, 2012).

Various serovars of leptospires have been found across Australia, associated with different host species. In the north of Australia including the Top End, serovar Australis is most commonly found and has been associated with a carrier status in rats and bandicoots (CCRRL, 2010). *Leptospira* serovar Australis infection causing clinical disease is seen in dogs in Darwin; however the maintenance host for these infections is not known (C Shilton, personal communication 2015). Much of the current information on leptospirosis in Australian native mammals does not relate to the serovars of *Leptospira* found in the NT. It is possible the common NT serovar (Australis) is more pathogenic to certain native mammals than presumed or that there are other serovars present in the NT that pose a disease risk for native mammals.

Serological evidence of leptospiral infection has been found in a wide range of Australian native mammals including brushtail possums, northern brown and other bandicoots, native rodents and dasyurids (Sullivan, 1974, Durfee and Presidente, 1979b, Durfee and Presidente, 1979c, Hathaway, 1981, Milner *et al.*, 1981, Slack *et al.*, 2006, Eymann *et al.*, 2007, Smythe *et al.*, 2007). Many infected animals do not show signs of disease however kidney, and occasionally liver, disease has been reported in wild possums, wild native rodents and other wild marsupials (Ladds, 2009). Bandicoots, brushtail possums and introduced rats may be maintenance carriers and may shed the bacteria in their urine (Eymann *et al.*, 2007), although the zoonotic risk to humans from brushtail possums carrying and shedding leptospires has not been quantified (Johnson and Hemsley, 2008). Disease from *Leptospira* infection has rarely been reported in any wildlife species, however in recent decades fatal outbreaks of leptospirosis have occurred in Californian sea lions (Wobeser *et al.*, 2001, Greig *et al.*, 2005). Eymann *et al.* (2007) investigated leptospiral infection in brushtail possums in the Sydney region and found, in addition to the expected serovars for which this species is known to be a maintenance host, evidence of infection with serovars for which brushtail possums are considered accidental hosts. Such serovars have the potential to cause serious disease in brushtail possums. *Leptospira* serovar Australis infection causing clinical disease is seen in dogs in Darwin; however the maintenance host for these infections is not known (C Shilton, personal communication 2015).

It is not known how long antibody levels persist in marsupial hosts after infection, however it is reported that the antibodies detected in the commonly used serological test for leptospiral infection may persist from weeks to months in brushtail possums (Durfee and Presidente, 1979a, Eymann *et al.*, 2007).

### 3.4.2.3 *Salmonella* spp.

*Salmonella* spp. are bacteria associated primarily with gastrointestinal infections. Infection is transmitted via the faecal-oral route and may be zoonotic. A wide range of vertebrate species, including humans, are known to become infected. *Salmonella* spp. are present throughout Australia although the serovars may vary according to geographic location. A very large number of serovars have been reported in Australia; all *Salmonella* serovars are considered potentially pathogenic (CFSPH, 2005, WHA, 2009).

Infection with *Salmonella* may cause life-threatening enteric and systemic disease. Many individuals carry *Salmonella* without signs of ill-health and may develop chronic carrier states and act as a reservoir for infection in other animals (Ladds, 2009, WHA, 2009). Exposure to novel or particularly virulent strains of *Salmonella* is more likely to result in disease (CFSPH, 2005). Seasonal variation in *Salmonella* occurs in both temperate and tropical areas, with higher prevalence shown in the summer or wet season (How *et al.*, 1982, Hart *et al.*, 1985).

*Salmonella* has been frequently cultured from free-ranging native mammals in Australia although infection is considered not to cause disease in the majority of cases (How *et al.*, 1982, Hart *et al.*, 1985, Ladds, 2009, WHA, 2009). Clinical disease, death or significant pathology, including outbreaks of salmonellosis have been reported in a wide range of Australian marsupials (including brushtail possums and bandicoots) and often in situations of captivity, high density or stress (Presidente, 1984, Ladds, 2009, WHA, 2009). Disease associated with *Salmonella* infection has not often been reported in native rodents. Immunocompromise may play a role in the development of clinical disease associated with *Salmonella* infection and disease is often related to concurrent diseases. If *Salmonella* is present in wild populations, animals may be at increased risk when other diseases become active in the population, thus an understanding of prevalence (and strains) of *Salmonella* within wildlife populations is important.

## 3.4.3 Protozoa

### 3.4.3.1 *Babesia* spp. and *Theileria* spp.

*Babesia* and *Theileria* are genera of protozoa that parasitise the red blood cells (haemoprotozoa) of a wide range of mammals, including a wide range of Australian native mammal species (Ladds, 2009, WHA, 2011b). They both have a worldwide distribution and the common method of transmission is via ticks. Babesiosis is an important disease of livestock and can be potentially fatal. Signs of infection in livestock include anaemia and systemic illness (Kocan and Waldrup, 2001).

Although *Babesia*, *Theileria* and other piroplasms are recognised in a wide range of Australian native mammal species, information is limited and there is little knowledge of the clinical effects of piroplasm infection in Australian mammals. Previous studies have reported *Babesia* spp. in bandicoots, antechinus, quolls, echidnas, bettongs and other macropods. Most reports of infection have not been associated with disease; however there are occasional reports of significant clinical disease associated with *Babesia* spp. infection in Australian species with clinical disease more likely to occur in immunosuppressed individuals (Ladds, 2009, Paparini *et al.*, 2010, WHA, 2011b, Paparini *et al.*, 2012).



In the brown antechinus (*Antechinus stuartii*), *Babesia* infection has been associated with moderate anaemia (Cheal *et al.*, 1976) and heavy infection was highly prevalent in moribund individuals undergoing post-mating mortality (Barker *et al.*, 1978). Depression and anaemia has also been observed in an eastern grey kangaroo (*Macropus giganteus*) with a significant *Babesia* infection (Ladds 2009). *Babesia* spp. have previously been identified in northern quolls and in bandicoots, including the northern brown bandicoot, with no associated signs disease (Mackerras, 1959, Obendorf, 1993, Bangs, 1996).

*Theileria* spp. infection has been described in bandicoots including northern brown bandicoots, bettongs and other macropods but infection is apparently unrelated to disease (Clark, 2004).

### 3.4.3.2 *Hepatozoon* spp.

*Hepatozoon* spp. are haemoprotozoan parasites which infect a wide range of mammalian and other vertebrate species and are poorly host specific. Infection is generally spread by biting arthropods, particularly ticks (Smith, 1996). *Hepatozoon* infections in dogs can cause significant systemic disease; the recently emerged *H. americanum* causes fatal disease in dogs in northern America (Chomel, 2011) which highlights the potential threat of *Hepatozoon* to naïve populations. Infection with *Hepatozoon* has been reported in a range of Australian native mammals including quolls, possums and bandicoots, generally without associated signs of disease (Mackerras, 1959, Bettiol *et al.*, 1996, Ladds, 2009), however knowledge on the significance of *Hepatozoon* infection in Australian native mammals is limited.

### 3.4.3.3 *Toxoplasma gondii*

*Toxoplasma gondii* is a protozoal parasite of the coccidian family with a global distribution. The definitive hosts are felids, primarily the domestic cat. A wide range of mammals may act as intermediate hosts. Australian marsupials (particularly macropods, bandicoots and wombats) are well recognised as having heightened susceptibility to *Toxoplasma* infection, with significant clinical disease and mortality being observed in both captive and free-ranging Australian marsupials (Dubey and Odening, 2001, Ladds, 2009, Portas, 2010). It is thought that this is due to the fact that they evolved in the absence of cats, and hence were not exposed to toxoplasmosis until recent years. Rodents in general are also considered susceptible to infection but less commonly develop clinical disease (Dubey and Odening, 2001).

After infection, cats shed infective cysts in their faeces; these may survive in moist soil for months to years. Contamination of soil by cat faeces and subsequent ingestion of material contaminated with soil-borne cysts (or possibly contaminated water) is the method by which wildlife are thought to become infected. Australian wildlife may develop clinical disease soon after infection, or may develop sub-clinical disease which may recrudesce (re-emerge) at a later stage, if factors cause immunocompromise. Such factors may include concurrent disease, nutritional, climatic or reproductive stressors, age, translocation or predatory pressures. Young animals are probably more susceptible than adults to infection and disease (Dubey and Odening, 2001). There is some evidence that *Toxoplasma*-infected dams may be able to pass *T. gondii* infection to their offspring *in utero* or via milk (Parameswaran *et al.*, 2009), however the significance of these forms of transmission is not fully understood, and it is likely that the greatest risk to Australian wildlife in acquiring toxoplasmosis is environmental contact with *Toxoplasma* cysts shed by cats (Hill and Dubey, 2002, Portas, 2010).



*Toxoplasma* infection affects multiple body systems. Clinical signs are seen in the respiratory, muscular and nervous systems (Dubey and Beattie, 1988). It has been hypothesised that central nervous system effects of toxoplasmosis may change the behaviour of infected individuals in subtle but significant ways. Studies have found increased risk-taking behaviour in humans with evidence of *Toxoplasma* infection in the brain (Webster, 2007). Toxoplasmosis may change behaviours in small wildlife species; for example bandicoots may exhibit uncharacteristic docility, activity during daylight hours, apparent blindness, lack of coordination, and loss of ability to seek shelter and this may reduce predator-avoidance behaviours, with a result that diseased animals are more readily predated (Obendorf and Munday, 1990, Lynch, 2008, Hollings *et al.*, 2013). Infected rodents have been shown to have decreased aversion to the smell of feline urine. The loss of this predator-avoidance behaviour may lead to a resultant increase in risk of predation (Berdoy *et al.*, 2000, Kaushik *et al.*, 2014).

Studies have looked at the seroprevalence of *Toxoplasma* infection in free-ranging Australian wildlife and the evidence for clinical disease in different species (Obendorf *et al.*, 1996, Eymann *et al.*, 2006, Hill *et al.*, 2008, Hollings *et al.*, 2013, Fancourt *et al.*, 2014). As with many other diseases, individuals may exhibit a seropositive state but not show evidence of clinical disease. A wide range of species have shown serological evidence of *Toxoplasma* infection. Toxoplasmosis, resulting in significant disease and death, has been described in a number of Australian marsupial species including bandicoots (Desmonts and Remington, 1980, Obendorf *et al.*, 1996), kowari (*Dasyuroides byrnei*) (Attwood *et al.*, 1975), brushtail possums and native rodents (Ladds, 2009). Species that commonly feed on the ground, including those that ingest soil, appear to be most susceptible to infection and disease. Bandicoots are recognised as being highly susceptible to the disease; evidence suggests that some infected bandicoots die quickly after infection, but a significant proportion survive initial infection with *T. gondii* (Lenghaus *et al.*, 1990).

Dasyurids appear more resistant to *Toxoplasma* infection and related disease than other marsupial groups (Attwood *et al.*, 1975, Holz, 2008, Ladds, 2009, Fancourt *et al.*, 2014). An earlier review surmised that there was no evidence for clinical disease from toxoplasmosis in free-ranging dasyurids (Obendorf, 1993), however clinical disease has since been reported in captive dasyurids (Holz, 2008).

It is hypothesised that seropositive animals of susceptible species live shorter lives, perhaps due to increased risk of predation as a result of behavioural changes and studies have compared indicators of survival in seropositive and seronegative Australian wildlife. Seropositive animals of some species (bandicoots, possums) were recaptured less often than seronegative individuals which suggests that, at least in some marsupials species, free-living individuals infected with *T. gondii* do not survive for long periods of time (Obendorf *et al.*, 1996, Eymann *et al.*, 2006). Conversely Fancourt *et al.* (2014) found that *Toxoplasma* seropositivity did not reduce longevity in eastern quolls (*Dasyurus viverrinus*).

The opportunities for a free-ranging marsupial to be exposed to, and acquire *Toxoplasma* infection will be influenced by the density of feral cats, the proportion of juvenile cats in the population (as juvenile cats generally shed cysts in vast quantities), the size of the home range of the individual marsupial (presuming animals with larger home ranges have more opportunities to encounter infective cysts in the environment) and the environmental conditions (moist, temperate conditions favour cyst survival) (Dubey and Odening, 2001).

There is little existing information about the presence and prevalence of *Toxoplasma* in the Top End, other than the study undertaken by Oakwood and Pritchard (1999), which found no histological and little serological evidence of *Toxoplasma* in carcasses of northern quolls. Persistence of antibodies to *Toxoplasma* in Australian mammals will be partly dependent on the type of serological test used and the type of immunoglobulin measured. Immunoglobulin G, measured in the commonly used MAT serological test, persists for prolonged periods of time in most mammals and possibly for life (Hill and Dubey, 2002).

### 3.4.3.4 Trypanosomes

Trypanosomes are protozoan parasites found in the blood of infected animals. There is an enormous variety of trypanosomes and they infect a wide range of vertebrates with varying effects; some cause significant disease and some are non-pathogenic (WHA, 2011c, Desquesnes *et al.*, 2013). An invertebrate vector (usually a biting insect) is required to transmit the parasite (Averis *et al.*, 2009).

Many trypanosomes are 'host specific' (e.g., they are co-evolved with their host and do not cause disease). Seemingly benign trypanosomes may cause disease if they are introduced to a naive population of animals. For example, the extinction of the native Christmas Island rodent, MacLear's rat (*Rattus macleari*) has been associated with the introduction of a trypanosome carried by *R. rattus* (Wyatt *et al.*, 2008). It has also been hypothesised that the now exotic *T. lewisi* may have played a role during the fauna decline identified in Australia between 1875 and 1925 (Abbott, 2006).

Animals infected with trypanosomes may show no signs of disease or may exhibit a range of haematological or systemic signs; infection can be fatal. Exotic trypanosomes (those not occurring in Australia) affecting domestic animals and humans often result in anaemia, as well as other systemic signs of disease, and have been shown to cause immunosuppression in the host (Onah *et al.*, 1998, Connor and Van den Bossche, 2004, Dargantes *et al.*, 2005). Trypanosome infections have been reported in a wide range of Australian native mammals and are generally considered asymptomatic however in many cases the implications of infection have not been comprehensively studied (Averis *et al.*, 2009, Thompson *et al.*, 2014). Recently, trypanosome infection has been associated with the decline of the woylie (Botero *et al.*, 2013) and possible disease, including anaemia, in koalas (McInnes *et al.*, 2011). Exotic trypanosomes such as *T. evansi* (Surra) in South East Asia would have serious health implications, should they enter Australia (Reid *et al.*, 2001, Desquesnes *et al.*, 2013). Trypanosome infection and disease in Australian native mammals has been recently reviewed by Thompson *et al.* (2014).

Trypanosome infection has been reported in a range of bandicoots, dasyurids and in brushtail possums, however infection was not found in a small number of wild northern quolls studied in Qld (Averis *et al.*, 2009, McInnes *et al.*, 2011, Paparini *et al.*, 2011, WHA, 2011c, Botero *et al.*, 2013, Thompson *et al.*, 2014). Infection has also been described in a range of native rodents (Ladds, 2009, WHA, 2011c, Thompson *et al.*, 2014).

### 3.4.3.5 Protozoal endoparasites

*Giardia* spp. are protozoal parasites of the GIT and infection is spread through environmental contamination with infected faeces, then subsequent ingestion of infective cysts. Infection has been detected in a range of Australian species including brushtail possums, bandicoots and dasyurids (Ladds, 2009). There is little current information on the strains occurring in free-ranging Australian wildlife nor on the significance of this infection in Australian mammals, however *Giardia* spp. are considered potential pathogens in all mammal species including native Australian mammals. It is believed that many infections in wildlife involve *G. duodenalis*, suggesting a human origin for the infection (Thompson *et al.*, 2010a).

Recent studies of *Giardia* spp. in WA wildlife showed low to medium prevalence across a range of host species (Paparini *et al.*, 2010, Thompson *et al.*, 2010a) and an earlier study found a medium prevalence of *Giardia* spp. in Tasmanian wildlife, including eastern barred and southern brown bandicoots (Bettiol *et al.*, 1997). *Giardia* spp. infection in free-ranging wildlife may have impacts on domestic animal and human health (Bettiol *et al.*, 1997, CFSPH, 2012).

*Cryptosporidium* spp. are protozoal parasites of the GIT, transmitted via the faecal-oral route, that may cause disease in a range of mammals (Ryan and Power, 2012). Infection in wildlife is considered to be mostly asymptomatic but clinical signs, when seen, include diarrhoea. Infection and disease is associated with stress, age and other factors influencing immune competence, and may also be influenced by season (Ladds, 2009). Wildlife host-adapted species of *Cryptosporidium* are likely to have evolved in close association with marsupials and are thought unlikely to result in disease (Appelbee *et al.*, 2005).

In taxa of interest to our study, *Cryptosporidium* spp. have been reported in western barred, southern brown and long-nosed bandicoots (*Perameles nasuta*) and brushtail possums (Ryan and Power, 2012), and in antechinus (Ladds, 2009). In NZ, *C. parvum* has often been linked to outbreaks of diarrhoea in captive brushtail possum (Ladds, 2009). In many studies, *Cryptosporidium* spp. positive wildlife were inhabiting areas associated with humans and it is possible that *Cryptosporidium* oocysts from the environment were passively passing through the GIT of positive animals. Rodents including black rats and other species have been reported as hosts for *Cryptosporidium* spp. (Ryan and Power, 2012). Feral pigs may also be involved in the epidemiology of the pathogen (Ryan and Power, 2012), which may be a consideration for the Top End of the NT.

### 3.4.4 Helminth endoparasites

There are many reports of helminth parasites in our target taxa, however most reports do not associate the presence of endoparasites with disease. This review is limited to those helminth parasites, in target taxa, which have been associated with signs of disease, either microscopically or clinically, in a non-captive setting. The information below has been summarised from Ladds (2009) and Clark (2004).

#### 3.4.4.1 Cestodes (tapeworms)

Here we report only on cestodes associated with disease in target taxa. **Spargana** (larval stages of *Spirometra* spp. and *Diphyllbothrium* spp. tapeworms) have been reported in a wide range of Australian mammals including northern quolls, northern brown bandicoots and rodents. Infection is mostly sub-clinical although some signs of disease have been reported and some deaths in antechinus spp. have been attributed to this infection.

**Anoploetaenia dasyuri** may cause disease in brushtail possums; **Dasyurotaenia** spp. have been reported in Tasmanian devils and quolls; association with disease is not clear. **Bertiella trichosuri** is reported in brushtail possums; heavy burdens may result in reduced body condition but are otherwise not considered highly pathogenic.

#### 3.4.4.2 Nematodes (roundworms)

Here we report only on nematodes associated with disease in target taxa. **Filaroid nematodes** from the family *Onchocercidae* are found in a variety of anatomical sites in Australian mammals, including blood vessels, abdominal cavity, subcutaneous tissues, tendons and skeletal muscle. Filaroid nematodes have a blood-sucking arthropod intermediate host (e.g., ticks, biting flies and mosquitoes). The adult worms produce larvae (not eggs) that circulate in the blood stream (microfilariae) or accumulate in the skin. Presence of the larval forms in the blood is indicative of the presence of adult forms in various solid tissues in the host's body. Microfilariae from the genera *Breinlia*, *Cercopithifilaria*, *Dirofilaria*, *Pelictus* and *Sprattia* have been described in Australian mammals, including native rodents. Some species have been associated with disease in the marsupial host (Clark, 2004).

***Parastrongyloides* spp.** have been reported in bandicoots and brushtail possums; associated with disease and possibly death; ***Trichostrongylus* spp.** in brushtail possums may cause disease if burdens are heavy; ***Baylisascaris* spp.** in spotted tail quolls may cause muscle damage; ***Capillaria* spp.** are commonly found in the tissues of many Australian mammals with only occasional evidence of disease. However, liver disease associated with *Capillaria* spp. infection has been seen in brushtail possums. ***Ophidascaris* spp.** have caused liver lesions, but no associated clinical signs in common or mountain brushtail possums, and have been tentatively linked to disease and death in antechinus. ***Physaloptera* spp.** in large numbers may cause GIT disease in eastern barred bandicoots; ***Sprattia* spp.** have caused liver lesions in mountain and common brushtail possums and northern quolls. ***Angiostrongylus* spp.** (the rat lung worm) is a fairly recent arrival in Australia with its hosts, brown and black rats. The migrating larvae may cause CNS disease in vertebrate hosts including native rodents and brushtail possums. Distribution appears to be limited to Qld and NSW coastal areas. ***Breinlia* spp.** occur in the body cavity of quolls, brushtail possums and rodents and may cause lesions; ***Cercopithifilaria* spp.** infest a number of Australian mammals including rodents and bandicoots and cause disease of the eye and subcutaneous tissues; ***Marsupostrongylus* spp.** occur in the respiratory tract of many marsupials including dasyurids, bandicoots and possums. Disease is rare but has been reported in brushtail possums, eastern barred bandicoots and *Antechinus* spp.; ***Gallegostrongylus* spp.** has been reported in native and feral rodents but only in NSW. The parasite has caused clinical disease in the swamp rat (*Rattus lutreolus*). ***Trichinella pseudospiralis*** has been seen in quolls and other Australian native mammals and is suspected to occur in brushtail possums. It has been associated with disease in quolls.

### 3.4.4.3 Trematodes (flukes) and Acanthocephalans (thorny headed worms)

Trematodes are rarely reported in Australian native mammals and are very seldom associated with disease. *Fasciola hepatica* has been reported in brushtail possums and may result in liver damage but is not present in the NT. A range of thorny headed worms has been reported in bandicoots but most often without associated disease, unless burdens are heavy. A range of pentastomid species (worm-like parasites related to arthropods) has been seen in native rodents; northern quolls and other dasyurids; and northern brown and other bandicoots with no reports of related disease.

### 3.4.5 Ectoparasites

Ectoparasites have a potential role as vectors of pathogens including viruses, bacteria and protozoa. In addition, heavy burdens of some ectoparasites can cause significant disease in their own right. This review is limited to ectoparasites reported to cause disease in the target taxa.

**Mites:** Sarcoptic mange (*Sarcoptes* spp.) has been reported (but is not considered common) in bandicoots, brushtail possums and dasyurids. Mange as a result of demodex (or related) mites has been reported in dasyurids and other native mammals, however only in a captive setting. Trombiculid mites are commonly reported and are seen as orange patches of mites on the skin (especially on the inner thighs) and may result in skin lesions and moist dermatitis in a range of species. Dermatomyssid mites have been associated with dermatitis in brushtail possums. *Dasyuochirus* and *Myocoptes* spp. mites have been associated with extensive mange lesions in spotted tail quolls.

**Ticks:** As well as acting as vectors for infectious disease, tick infestation may result in paralysis or blood loss sufficient to cause anaemia. Tick-associated anaemia and poor growth has been reported in juvenile northern brown bandicoots. Paralysis ticks (*Ixodes holocyclus*, *I. hirsti* and *I. cornuatus*) do not occur in the NT. Ticks may also cause skin damage and irritation and allow ingress of localised bacterial infections. Scrotal lesions have been seen in brushtail possums with heavy tick infestations.

**Fleas:** Heavy flea burdens may contribute to ill health in free-ranging mammals, including brushtail possums, where rump wear may be associated with flea burdens, amongst other factors. Individuals with heavy flea burdens are often immunocompromised and may have concurrent disease processes occurring. Very heavy flea burdens may result in anaemia. Dasyurids are reported to host a species of fleas where flea larvae develop inside the skin of the host, resulting in characteristic skin nodules.

**Lice:** Heavy lice burdens may be associated with skin disease and irritation in Australian mammals.

## 3.5 Ancillary tests and investigations of relevance to our study

### 3.5.1 Haematological analyses

Peripheral blood consists of a variety of blood cells, and serum (the liquid fraction). Analysis of a blood sample (called haematological analysis or a complete blood count [CBC]) looks at the numbers of red blood cells, white blood cells and platelets and describes the type and nature of each class of blood cell. It also looks at the levels of protein in the serum. Such tests can provide a valuable insight into the health of an individual and hence a population. In particular, information is gained on the degree, and type of anaemia (if present) and how the body is responding to infection or other inflammatory challenges (Clark, 2004). Complete blood counts require specialised equipment and can only be performed at an established lab on freshly collected blood. A limited haematological analysis (packed cell volume [PCV] and estimation of total plasma or serum protein [TPP]) can be performed at a field station.

### 3.5.2 Biochemical analyses

Analysis of enzymes, electrolytes and other products within the serum from a peripheral blood sample can be undertaken to gain further information on the health and physiological status of an individual (and hence a population). Biochemical analysis provides information on the health and functionality of various body systems including the liver, kidneys, skeleton and muscles. Together, the variables PCV, TPP, total white cell count (TWCC), albumin (Alb) and globulin (Glob) may be used as general indicators of the health of individuals and hence of populations (Stockham and Scott, 2013).

### 3.5.3 *De novo* pathogen sequencing

*De novo* pathogen discovery testing may also be called next generation sequencing or deep molecular sequencing. It utilizes emerging molecular technologies to analyse biological samples for the presence of DNA and RNA from potential pathogens within the host. The molecular information is collated using powerful computer programs, to generate a list of organisms found within the biological sample. It is a potentially powerful tool, with the capacity to detect previously unknown or unrecognized pathogens (White *et al.*, 2014).

As opposed to traditional testing that targets a specific pathogen, *de novo* molecular sequencing creates a weighted list of potential agents (usually viral) that can be used to develop hypotheses for further investigation. Work may include targeted follow-up methods, such as PCR, to confirm any findings from agents of interest. Following this, traditional epidemiological approaches may be used to determine the significance and likely impact of any identified agents of disease on populations or individuals. The techniques are in their infancy, and techniques and protocols may not be fully refined and validated. Molecular sequencing can generate a very significant amount of raw data and appropriate interpretation of this data can be challenging. The technique requires delicate extraction of nucleic material from swabs which requires sterile lab conditions and experienced operators.



Samples need to be kept in ultra-cold (-80°C) conditions which can be challenging when working in remote locations. Both the processes of molecular sequencing and metagenomic analysis of the data are expensive and few laboratories in the Australasian region currently offer these services.

### 3.5.4 Stress studies

Glucocorticoid levels in fur and faeces can be used as an indirect assessment of stress levels in an individual over time, in contrast to levels in serum which will be reflective of the animal's most recent state of stress. Fur and faeces reflect glucocorticoid levels in the body over longer periods than the trapping event itself. Differences in glucocorticoid levels between individuals (or within an individual over time) may give insight into the impacts of medium to long term stressors such as predator presence, breeding seasonality, availability of resources or population densities (Sheriff *et al.*, 2011).

### 3.5.5 Studies of anti-oxidant capacity

The assessment of the anti-oxidative capacity of an individual is an emerging science, with the potential to provide valuable information about an individual's ability to cope with stressors in its environment (Rabus *et al.*, 2008, Schultz *et al.*, 2011). A commonly employed set of analyses, termed the oxidative stress index can be utilised in wildlife to determine the anti-oxidative capacity of an individual (Schultz *et al.*, 2011). Oxidative stress is a process implicated in a variety of cellular processes which can result in cell death (Evans and Cooke, 2004). Adverse situations, such as changes in environment, pressure from competitors and predators may cause an increase in oxidative stress, and hence reduce the animal's ability to cope with its environment on a cellular and physiological level. Variables measured include Vitamins A and E, ferric reducing ability of plasma, trolox equivalent antioxidant capacity, thiobarbituric acid reducing substances and ascorbic acid (Schultz *et al.*, 2011).

## 3.6 Trapping and sampling

A total of 281 individuals, including non-target species, were anaesthetised and sampled. At Cobourg Peninsula a total of 98 individuals were sampled; on Bathurst Island 68; Kakadu National Park 31; Darwin 33 and on Groote Eylandt 49 individuals. One feral cat was sampled from the Warddeken Indigenous Protected Area. Details of the locations surveyed, species (including non-target species) and genders of animals sampled are provided in Table 6. Brushtail possums were only trapped in reasonable numbers at Bathurst Island and Darwin; brush-tailed rabbit-rats were only trapped at Cobourg Peninsula and Bathurst Island and northern quolls were only trapped at Kakadu National Park and Groote Eylandt. Northern brown bandicoots were the only target species trapped in reasonable numbers at all major study locations.

Across all individuals sampled under anaesthesia there was a close-to-even sex ratio, with 160 (57%) males and 121 (43%) females with the majority (228; 81%) being adults. The close-to-even sex ratio of the sampled animals was in part achieved through a positive bias in selection of individuals trapped for anaesthesia and sampling, on a daily basis. In general, more males were trapped than females and the numbers sampled in part reflect this.

Non-invasive samples (ectoparasites, faecal samples) were collected from an additional 113 individuals trapped during additional routine DLRM fauna surveys (Table 7).

## 3.7 Health assessment

### 3.7.1 Health status by clinical examination

Of the individuals examined under anaesthesia, 264/281 (94%) were considered to be in normal health, as assessed by clinical examination. Seventeen individuals examined under anaesthesia (6%) were considered to be in abnormal health (4 brushtail possums; 2 brush-tailed rabbit-rats; 10 northern brown bandicoots; 1 northern quoll) and were across all locations. These 17 individuals were further assessed as either suffering from acute (n=8) or chronic (n=9) disease. Those with acute disease (1 brushtail possum; 1 brush-tailed rabbit-rat; 6 northern brown bandicoots) were suffering superficial injuries (assumed to be from conspecific aggression or minor trapping injuries; n=6) or serious or fatal injuries (from motor vehicle or predator trauma; n=2). Those assessed with chronic ill health (3 brushtail possums; 1 brush-tailed rabbit-rat; 4 northern brown bandicoots; 1 northern quoll) included individuals with significant skin disease, including chronic infected wounds and evidence of heavy ectoparasite infestation (further investigation is detailed below), and an emaciated juvenile (back young) brushtail possum from Bathurst Island with evidence of severe anaemia and a very high tick burden. Of the 17 individuals in abnormal health, skin disease formed a part, or a majority of the issues noted, for nine. Other than skin disease, no syndromes were consistently found in those individuals with abnormal health. Individuals with chronic ill health were from Bathurst Island (3 brushtail possums; 2 northern brown bandicoots); Cobourgh Peninsula (1 brush-tailed rabbit-rat; 2 northern brown bandicoots) and Groote Eylandt (1 northern quoll).



Table 6: Locations surveyed; species (including non-target species) and genders of animals sampled under anaesthesia.

F=female; M=male; BTP=brushtail possum; CON=brush-tailed rabbit-rat; NBB=northern brown bandicoot; NQ=northern quoll; BFTR=black-footed tree rat; AB=*Antechinus bellus*; MB=*Melomys burtoni*.

Location	BTP		CON		NBB		NQ		BFTR		Black rat		AB		Feral cat		MB		Grand Total
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	
Bathurst Is.	17	16	3	3	8	19			1	3	1	1							68
Cobourg Pen.	1	1	17	13	26	33			3	4			1		1	1		2	98
Darwin	3	5			9	7			3	3	2	2			1				33
Groote Eylandt					6	7	10	26											49
Kakadu Nat Park	1	1			1	9	12	8							1	1			32
Warddeken															1	1			1
Grand Total	21	23	20	16	50	75	22	34	4	6	1	3	1	1	1	2	1	1	281

Table 7: Summary of animals, by species and location, from which non-invasive samples were collected.

AB=*Antechinus bellus*; BFTR=black-footed tree rat; BTP=brushtail possum; CON brush-tailed rabbit-rat; DM=delicate mouse (*Pseudomys delicatulus*); MB=*Melomys burtoni*; NBB=northern brown bandicoot; NQ=northern quoll; RC=*Rattus colletti*; RT=*Rattus tunneyi*.

Sites	AB	BFTR	BTP	Feral Cat	CON	DM	MB	NBB	NQ	RC	RT	Grand Total
Bathurst Island			23					4			1	28
Cobourg	4	1			18		10	15			1	49
Peninsula												
Darwin		2	12				4	3			1	22
Kakadu Nat. Park				1		1	5		5	2		14
Grand Total	4	3	35	1	18	1	19	22	5	2	3	113

One adult male northern quoll from Groote Eylandt (G14021Q) was found dead in a trap one morning, after being trapped and anaesthetised two days earlier. The animal appeared healthy at the initial examination and was scored as in normal health at that time. At post mortem exam, there were no significant gross findings. Histopathology from tissues collected at post mortem indicated that the animal most likely died from cardiovascular collapse, potentially as a result of the stresses associated with being trapped three nights in a row. There was some evidence, upon examination of tissues, of possible toxic exposure to liver and kidney, although the pathologist commented that this was far from conclusive. PCV and TPP performed in the field at the time of anaesthesia were considered normal; no blood was collected for CBC at that time. Serum collected on day one was subsequently submitted for biochemical analysis, with no abnormalities noted except moderate increase in urea and CK. Serological and other diagnostic tests did not reveal evidence of any pathogens. It is not known why this quoll died in the trap. As there was no conclusive evidence of underlying disease, the factors leading to this animal's death remain uncertain.

### 3.7.2 Body condition and weights

The animals examined at all locations were generally in good body condition. A total of 229/281 (81%) animals had a body condition score (BCS) of 2.5 or above, considered in this study the cut-off for 'normal' condition. Of the 53/281 (19%) of animals assessed to be in sub-optimal condition (BCS below 2.5), 46 had a BCS of 2; 1 a BCS of 1.5 and 6 a BCS of 1. Body condition scores by site and species, with mean scores overlaid, are presented in Figure 4. When comparing BCS <2.5 across sites there were no significant differences. However when comparing BCS <2.0 across sites there was a significant location effect for lower BCS at Bathurst Island versus all other locations (OR=20.3, 95% CI: 2.4-172,  $p < 0.001$ ). Although 5 out of 6 of the individuals from Bathurst Island with BCS <2.0 were brushtail possums, the lower BCS for this species at this location was not significant when compared to brushtail possums from other locations (OR=4.3, 95% CI: 0.2-83,  $p=0.31$ ).

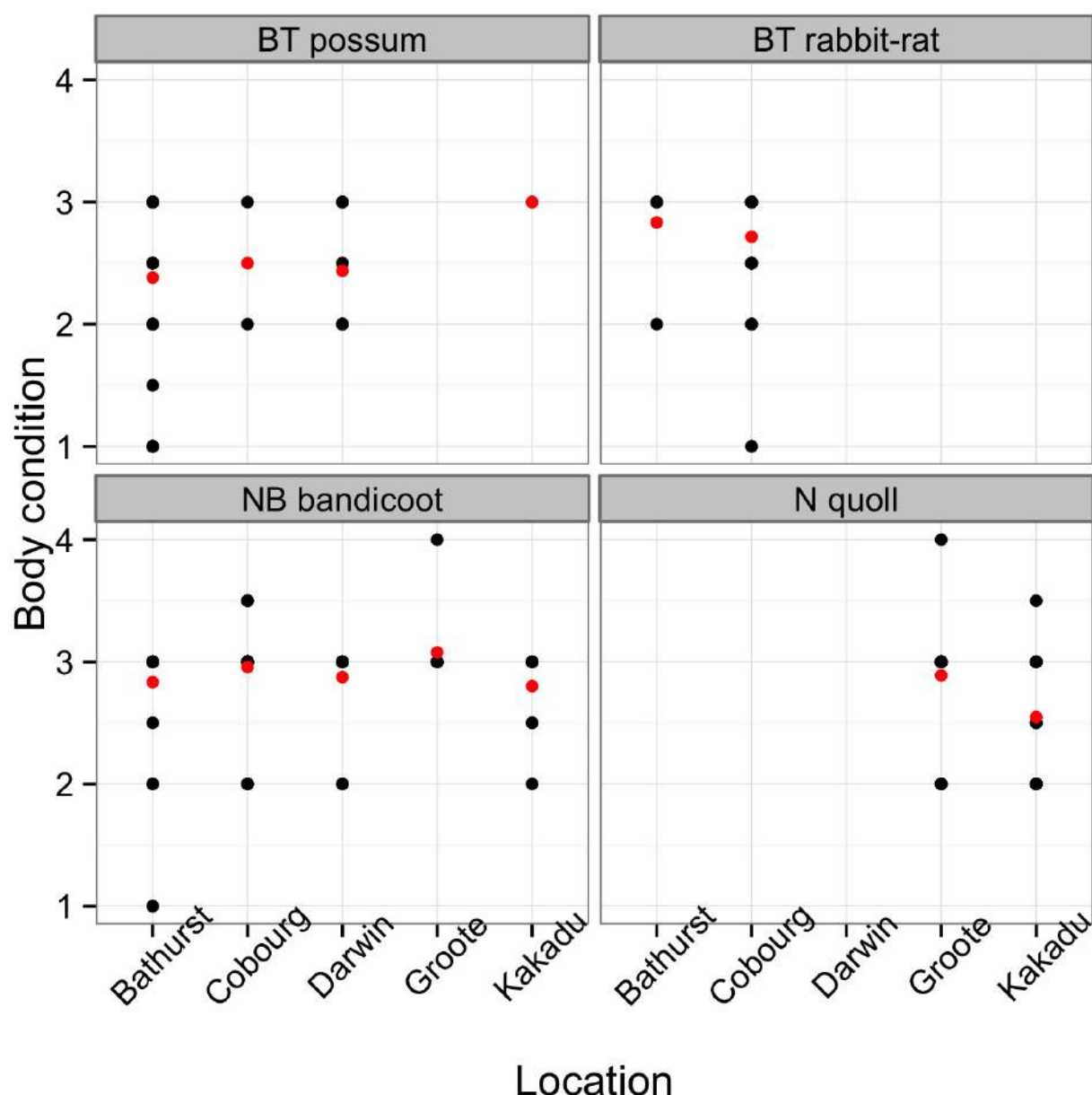


Figure 4: Range of body condition scores (BC, black dots) recorded from target species by location, with mean scores for each location indicated by red dots.

Note that each data point represents an aggregate of individuals.

Adult weights by species and sex (independent of health assessment or disease status) varied between locations (Figs. 5-8). Due to small sample sizes of sub-adults and juveniles these data are not presented.

Body weights for adult male and female northern quolls were significantly higher at Kakadu National Park compared with Groote Eylandt ( $p < 0.0001$ ). Adult male northern brown bandicoots were also significantly heavier at Kakadu National Park against all other locations sampled for this species ( $p < 0.0001$  for each site), except for Darwin. Male brushtail possums were significantly heavier in Darwin versus Bathurst Island ( $p = 0.003$ ).

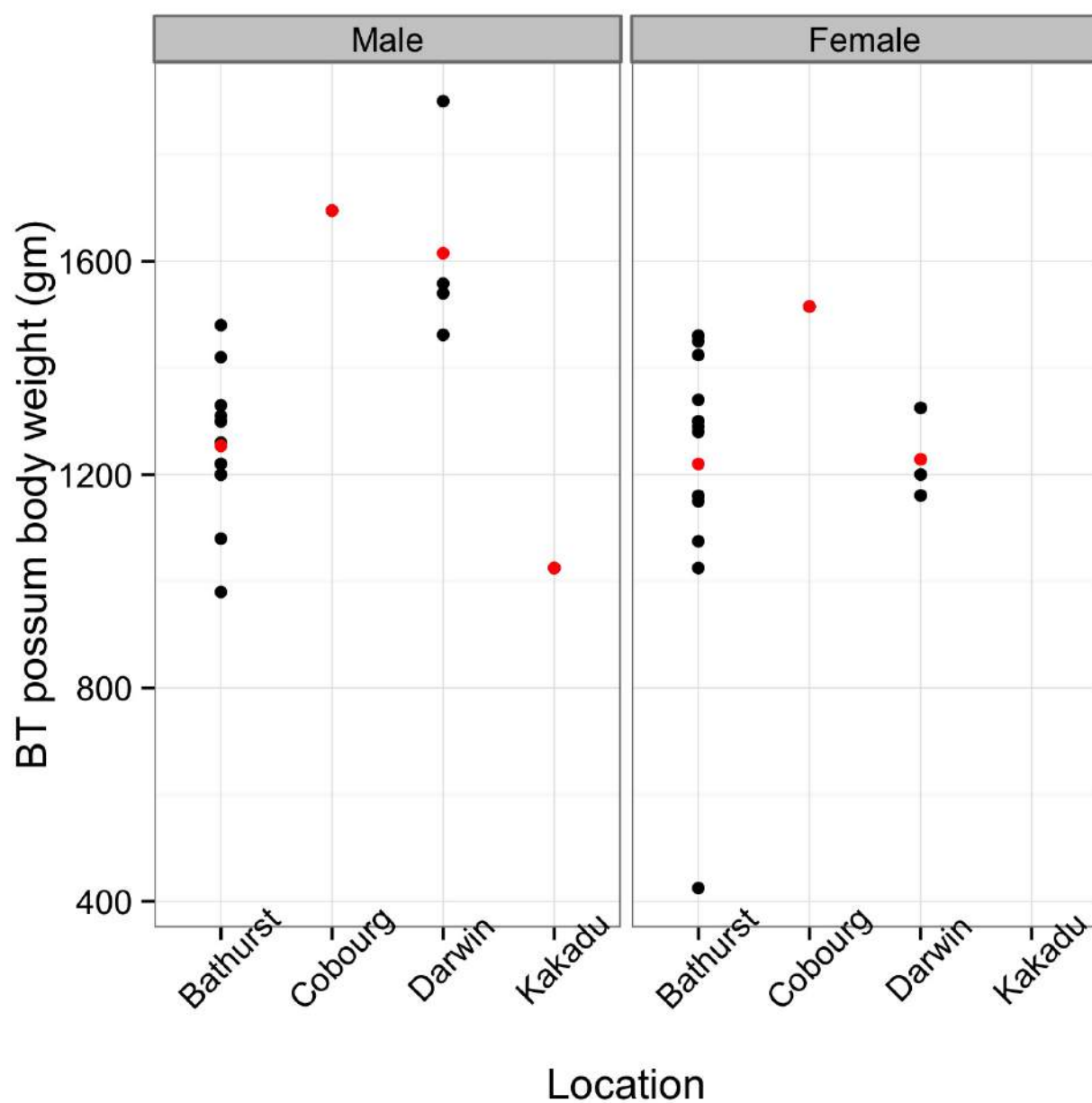


Figure 5: Weights for adult brushtail possums by location and sex, with mean weight overlaid as red dot.

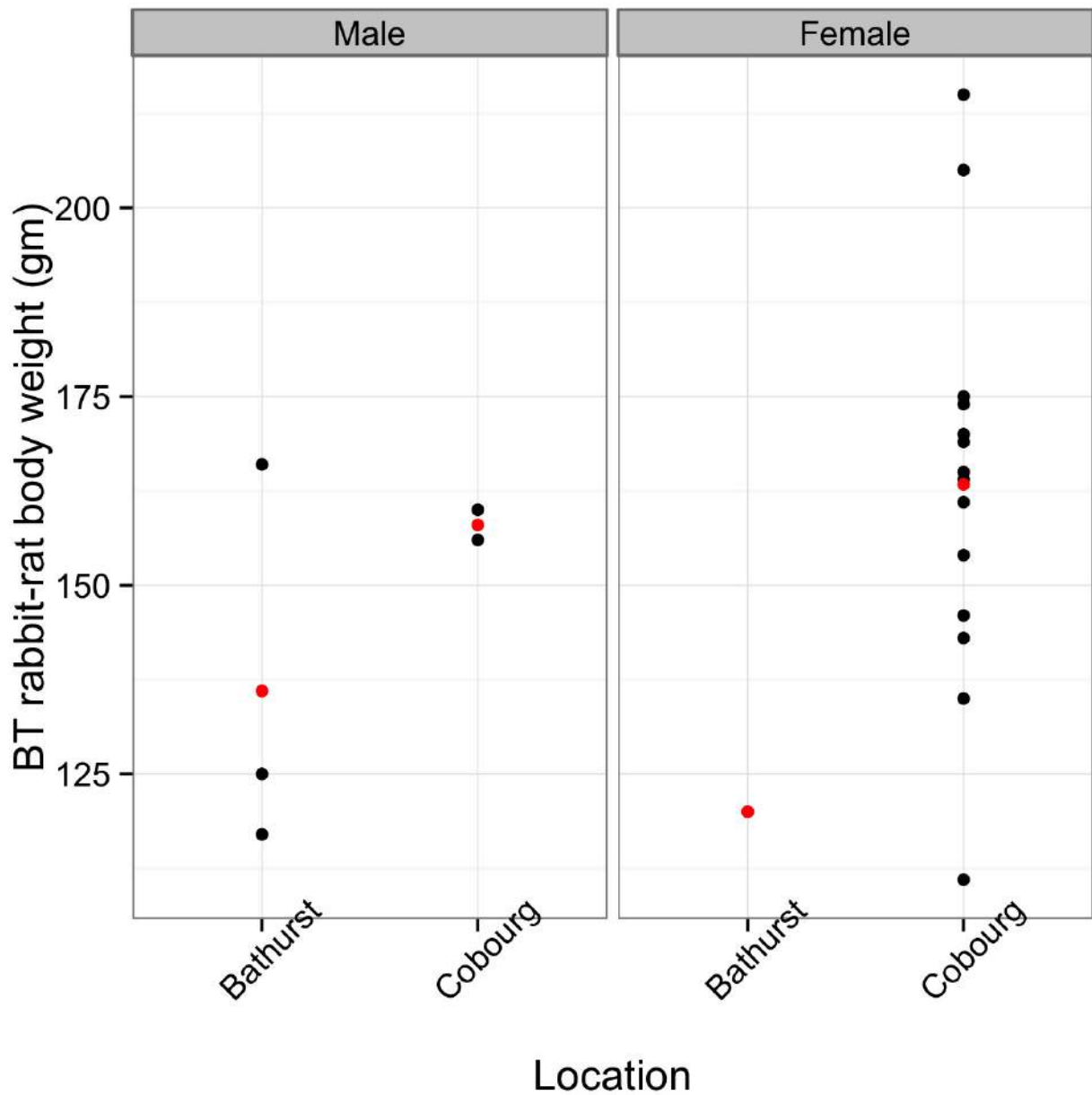


Figure 6: Weights for adult brush-tailed rabbit-rats by location and sex, with mean weight overlaid as red dot.

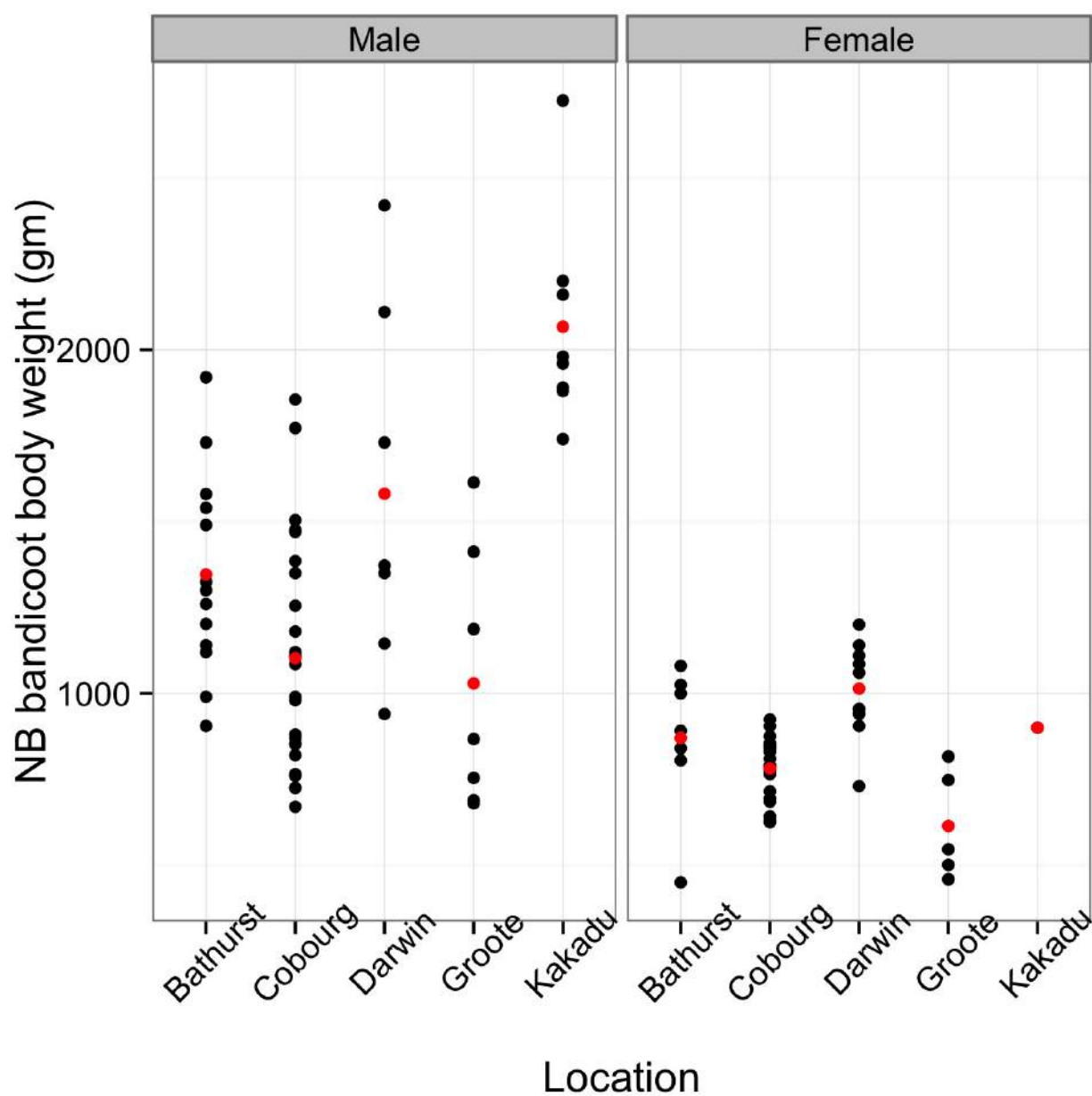


Figure 7: Weights for adult northern brown bandicoots by location and sex, with mean weights overlaid as red dot.

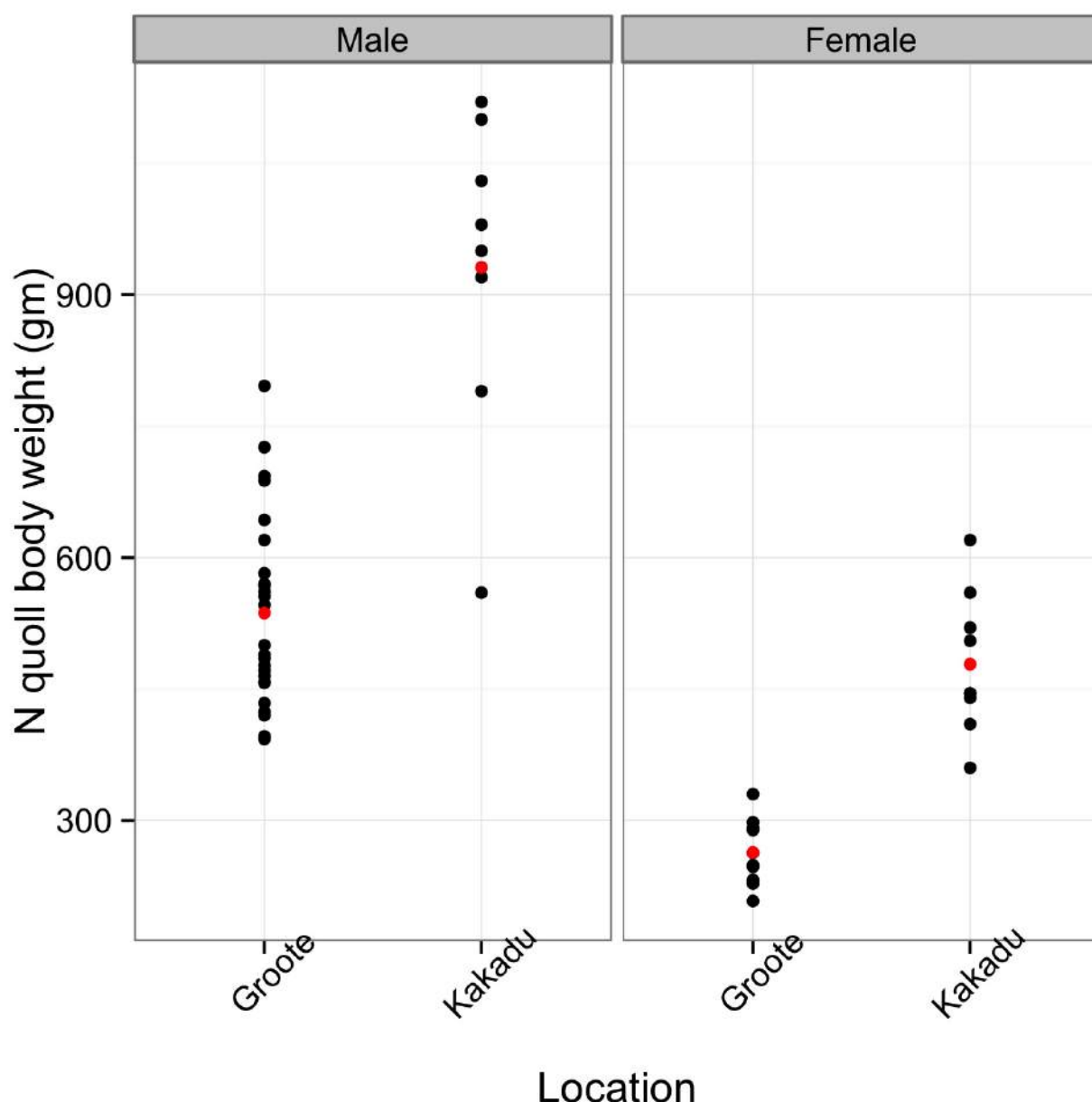


Figure 8: Weights for adult northern quolls by location and sex, with mean weights overlaid as red dot

### 3.7.3 Skin disease and ectoparasites

Ectoparasites were noted on 168/281 (60%) of animals examined. The majority of animals with ectoparasites (149/168; 89%) were judged to have a low burden. Ten percent (16/168) had a moderate burden and only three individuals (1%) were judged to have a heavy ectoparasite burden.

Ticks from the genus *Haemaphysalis* were present, primarily on bandicoots and possums, and were identified to species level (Table 8). *Haemaphysalis humerosa* was the most commonly identified tick and was found on brush-tailed rabbit-rats at Cobourg Peninsula and northern brown bandicoots at Bathurst Island, Cobourg Peninsula and Darwin. *Haemaphysalis ratti* was found on brushtail possums at Bathurst Island and Darwin; brush-tailed rabbit-rats at Cobourg Peninsula and northern brown bandicoots at Kakadu National Park. *Haemaphysalis bremneri* was found uncommonly on brushtail possums from Bathurst Island and brush-tailed rabbit-rats from Cobourg Peninsula. *Haemaphysalis novaeguinae* was identified from one northern brown bandicoot from Darwin and represents the first recording of this tick species in the NT, and the first ever recognition of immature stages of this tick.



Table 8: *Haemaphysalis* ticks identified on target species, June 2013–January 2014.

Ectoparasite species	NBB	BTP	Con	Total
<i>Haemaphysalis ratti</i>	1	5	2	8
<i>Haemaphysalis bremneri</i>	0	1	1	2
<i>Haemaphysalis humerosa</i>	32	0	3	35
<i>Haemaphysalis novaeguinae</i>	1	0	0	1
<b>Total</b>	<b>34</b>	<b>6</b>	<b>6</b>	<b>46</b>

Other parasites including mainly trombiculid mites were present on some individuals. Trombiculid mites (characterised by bright orange patches on the skin of the inner thighs and inguinal area), were seen in northern brown bandicoots from Bathurst Island, Cobourg Peninsula, Darwin and Groote Eylandt.

A range of skin lesions were seen in individuals at various locations, some associated with ectoparasites (Fig. 9). Results of histopathological investigations of skin disease are detailed below.



Figure 9: An image of the inguinal region of a male northern brown bandicoot with ectoparasite-related dermatitis.

Proliferative lesions were seen on the tail of one female brushtail possum from Bathurst Island (B14004P) (Fig. 10). Biopsies revealed diffuse papillomatous hyperplasia, consistent with papillomavirus infection. There were no inclusions to suggest involvement of a poxvirus.



Figure 10: Images of proliferative lesions on the tail of a brushtail possum from Bathurst Island.

Chronic, extensive dermatitis was seen in two adult northern brown bandicoots from Bathurst Island (B14036B & B14044B). Moderate to severe pyogranulomatous hyperplastic dermatitis with intra-lesional mites was seen in both cases, consistent with the clinical suspicion of reactions to trombiculid (or possibly dermanyssid) mites.

One adult female northern quoll on Groote Eylandt (G14041Q) had severe superficial focally ulcerative pyoderma with intra-lesional Gram positive cocci, possibly representing a case of primary bacterial pyoderma, for example due to *Staphylococcus* or *Streptococcus* spp. (Fig. 11).





Figure 11: Image of moist dermatitis on the ventral thorax of an adult female northern quoll from Groote Eylandt.

Rump wear was noted in 13/44 (30%) brushtail possums examined: 11/33 (33%) of brushtail possums from Bathurst Island; 0/2 at Cobourg Peninsula; 2/8 (25%) from Darwin and 0/1 from Kakadu National Park (Fig. 12). However comparing the odds of rump wear in brushtail possums at Bathurst Island against all other sites did not reveal a significant association (OR=2.25, 95% CI: 0.41-12.25,  $p=0.46$ ).



Figure 12: Image of moderate rump wear in a brushtail possum, showing loss of guard hairs on rump and tail base.

### 3.7.4 Haematological and biochemical indicators of health status

Comparing northern brown bandicoots in Darwin against all other sites, PCV was significantly higher at Cobourg Peninsula (mean=39.1,  $p=0.03$ ) and Kakadu National Park (mean=40.5,  $p=0.03$ ). No significant differences were found for PCV for other species (Fig. 13).



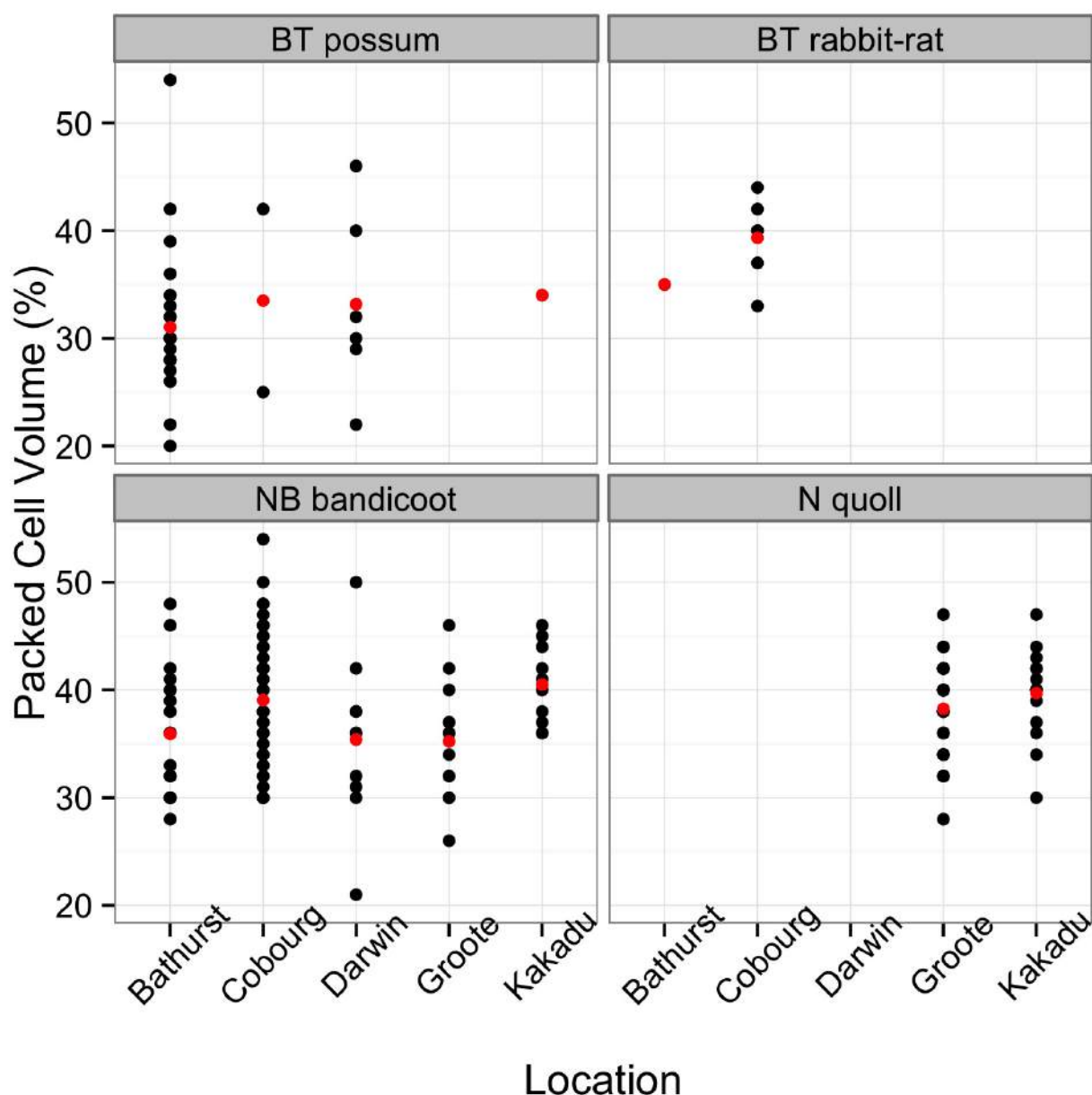


Figure 13: Packed cell volume (%) for location and species

TPP for brushtail possums located in Darwin was significantly higher (mean=75) than at Bathurst Island (mean=68,  $p=0.03$ ) (Fig 14). TPP for northern brown bandicoots in Bathurst Island was significantly higher (mean=65) than Cobourg Peninsula (mean=61,  $p=0.01$ ), Darwin (mean=60,  $p=0.03$ ), Groote Eylandt (mean=60,  $p=0.03$ ) and Kakadu National Park (mean=58,  $p=0.008$ ).

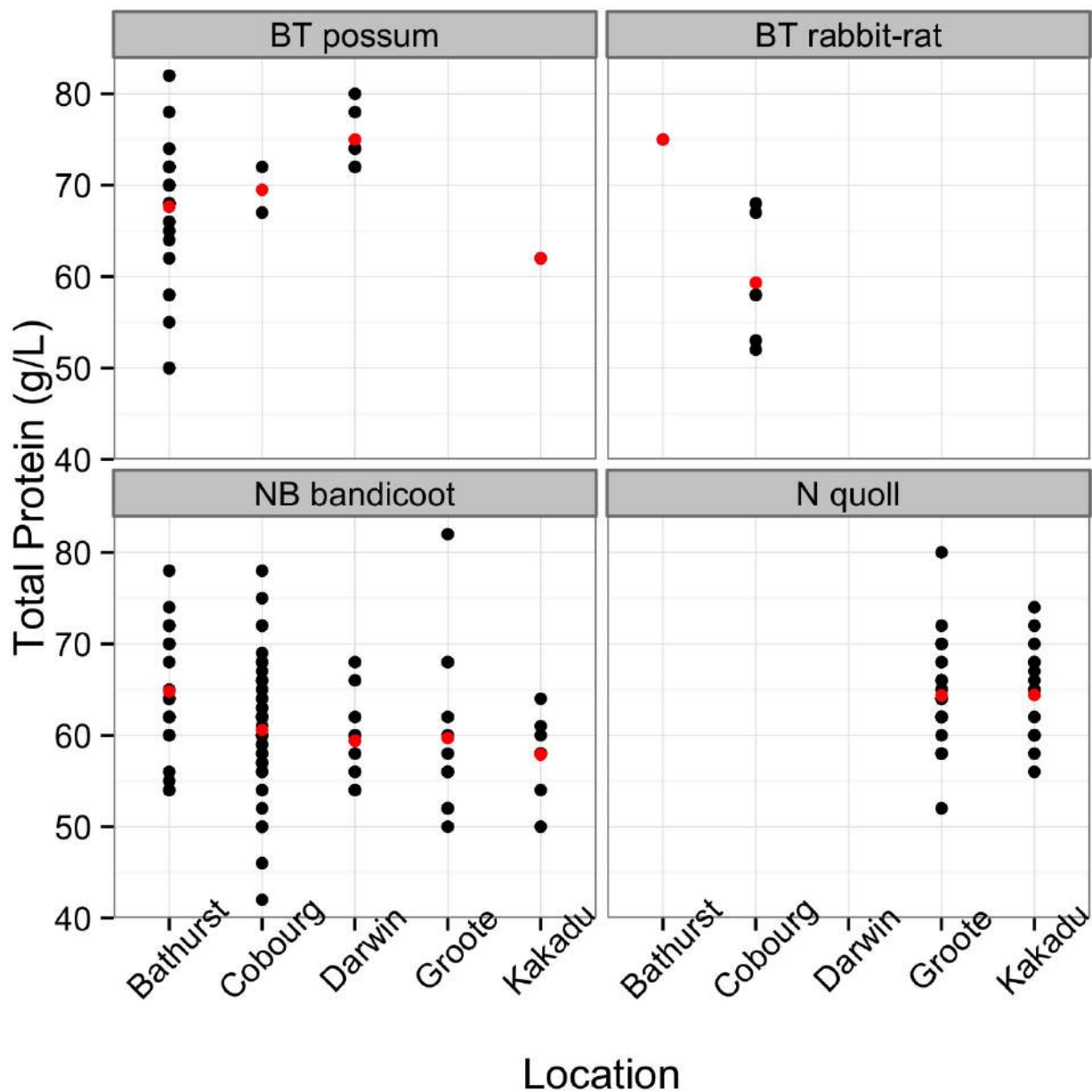


Figure 14: Total protein (g/L) for location and species.

Albumin for northern brown bandicoots at Kakadu National Park was significantly higher (mean=36) than at Bathurst Island (mean=30,  $p=0.03$ ), at Cobourg Peninsula (mean=30,  $p=0.02$ ) and at Groote Eylandt (mean=30,  $p=0.03$ ) (Fig. 15). No significant differences were found for globulin by location for each species (Fig. 16). No significant differences were detected for TWCC for each species by location (Fig. 17).

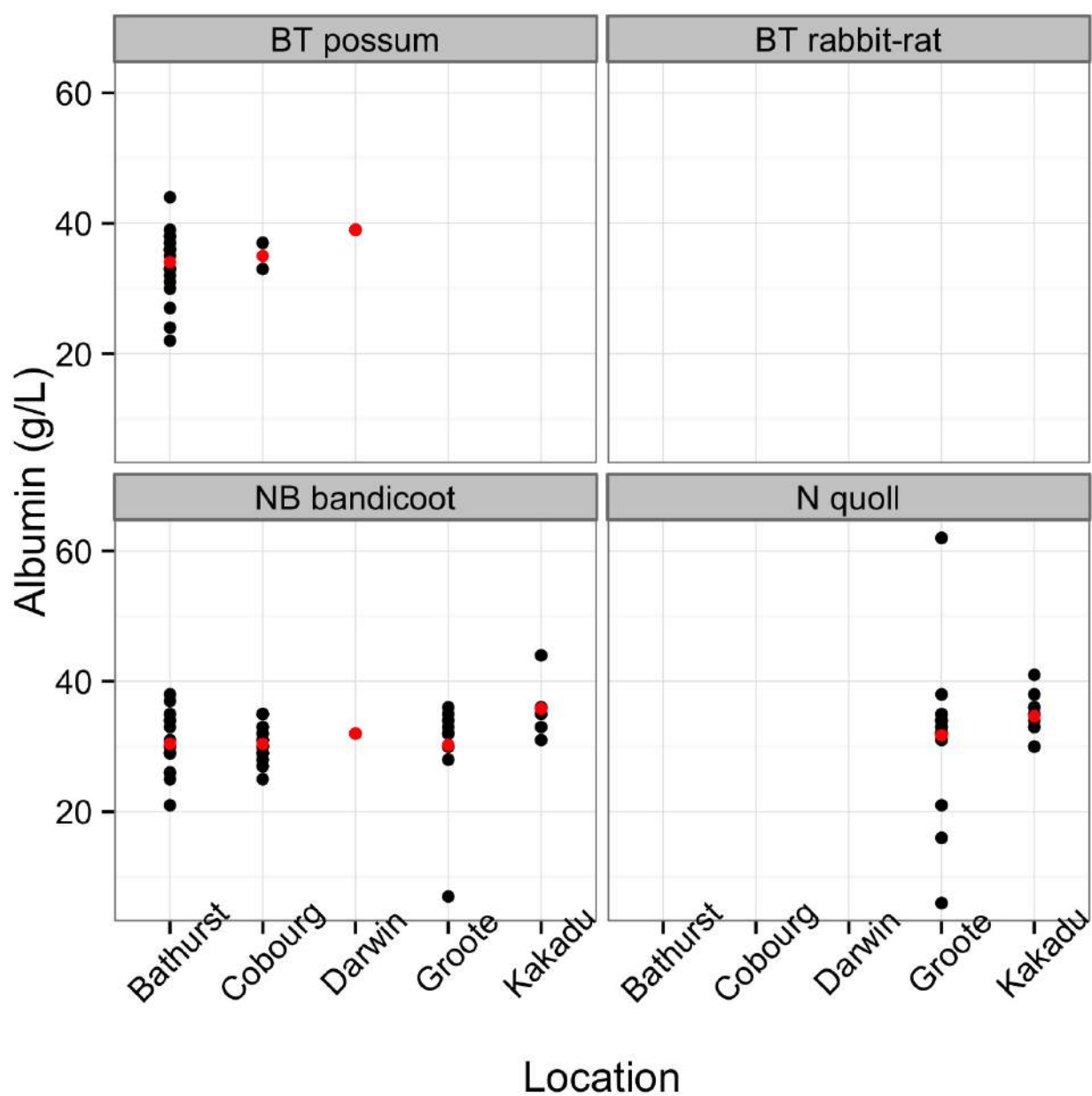


Figure 15: Albumin (g/L) for location and species.



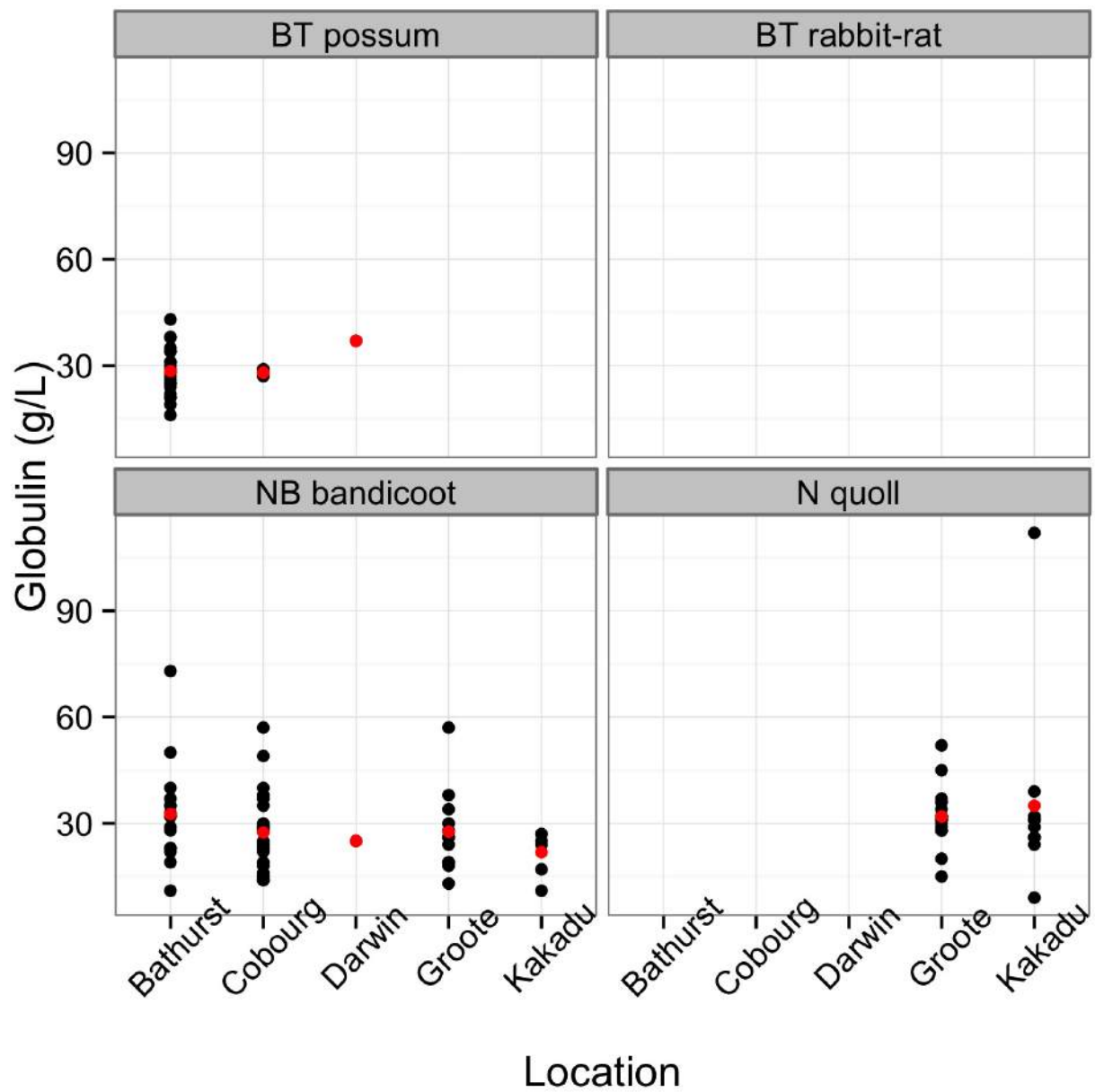


Figure 16: Globulin (g/L) for location and species.

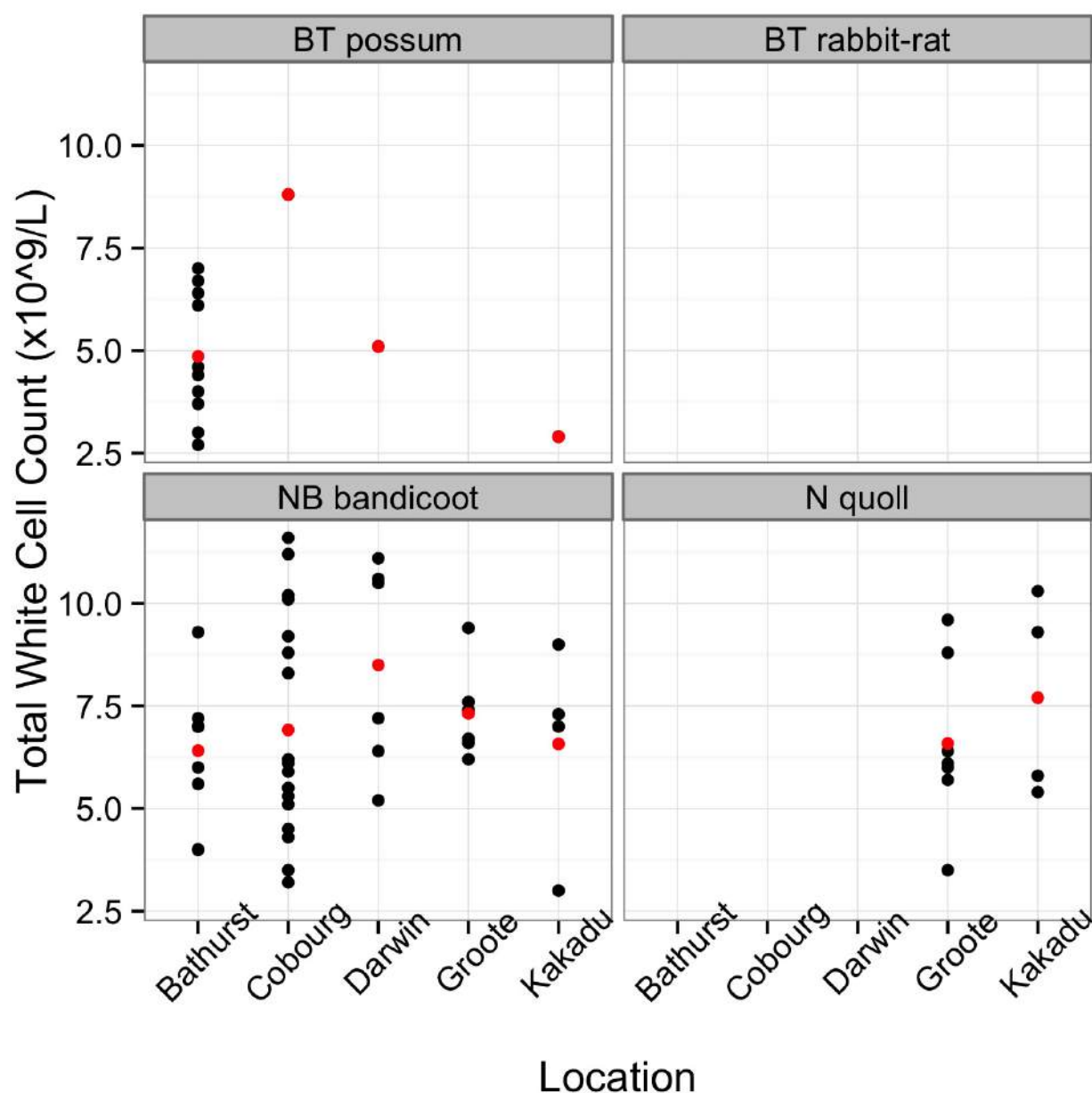


Figure 17: Total white cell count (x10<sup>9</sup>/L) for location and species.

Complete blood counts and biochemical profiles were performed on a sub-set of examined animals, governed largely by the ability to get fresh blood samples to the laboratory, ideally within 48 hr (CBC) and availability of sufficient serum (biochemistry profiles). Complete blood counts were performed on 72 individuals and biochemistry profiles on 128 individuals. Blood samples were extremely difficult to obtain from brush-tailed rabbit-rats and no blood samples of sufficient volume were collected to perform biochemistry or complete blood counts. Due to the limited volume of serum available for most individuals of other target species, not all biochemical analytes were tested in all individuals.

Complete blood counts were performed on 3 male and 1 female black-footed tree rats, 7 male and 6 female brushtail possums, 32 male and 12 female northern brown bandicoots and 8 male and 3 female northern quolls. Almost all individuals on which CBC was performed were assessed to be in normal health on the basis of physical examination under general anaesthesia.

Six individuals on which CBC were performed were assessed as being in abnormal health on clinical examination (2 northern brown bandicoots and 2 brushtail possums from Bathurst Island; 2 northern brown bandicoots from Cobourg Peninsula, all adults) with either acute or chronic physical abnormalities. Further analysis of haematological findings will be undertaken once reference ranges have been developed for the populations.

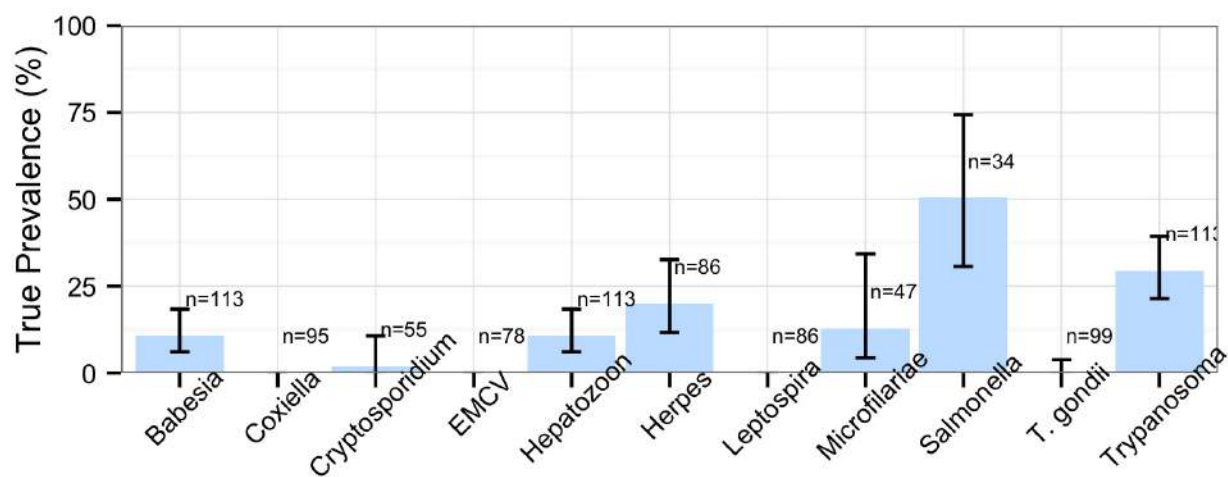
A total of 128 biochemical profiles were performed on 17 male and 11 female brushtail possums, 46 male and 18 female northern brown bandicoots and 27 male and 9 female northern quolls. Almost all individuals on which biochemistry profiles were performed were assessed to be in normal health on the basis of physical examination under general anaesthesia.

Ten individuals on which biochemical analyses were performed were assessed as being in abnormal health on clinical examination (3 northern brown bandicoots and 2 brushtail possum from Bathurst Island; 3 northern brown bandicoots from Cobourg Peninsula; one northern brown bandicoot from Kakadu National Park and one brushtail possum from Darwin, nine adults and one sub-adult) with either acute or chronic physical abnormalities. Further analysis of their biochemical findings will be undertaken once reference ranges have been developed for the populations.

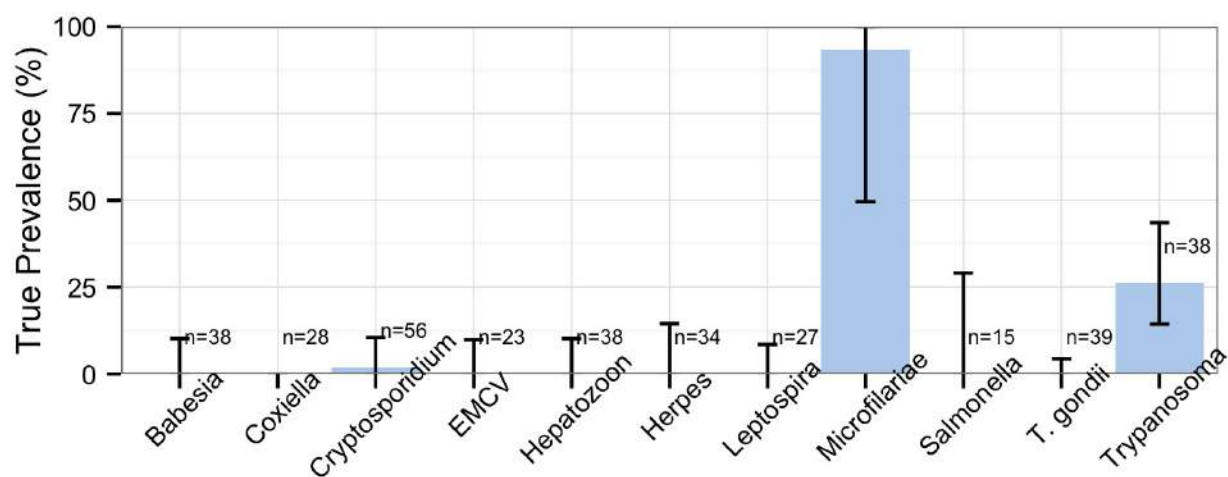
Elements of CBC and biochemical analysis (e.g., PCV, TPP, Alb and Glob) were analysed for associations with prioritised pathogens at the host species level. A more detailed analysis of CBC and biochemical profiles, including generation of suggested reference ranges for northern brown bandicoots (and potentially other target species), is planned and will be made available as a peer-reviewed publication.

### 3.8 Pathogen testing

A summary of true prevalence results for all pathogens tested by host species is presented (Fig. 18). A greater number of pathogens ( $n=7$ ) were detected in northern brown bandicoots, whereas only one pathogen (*Cryptosporidium* spp.) was detected, at a low prevalence, in the brush-tailed rabbit-rat. Sample sizes in the brush-tailed rabbit-rat were too small to provide statistical confidence of presence or absence for most pathogens.



NB bandicoot



BT possum

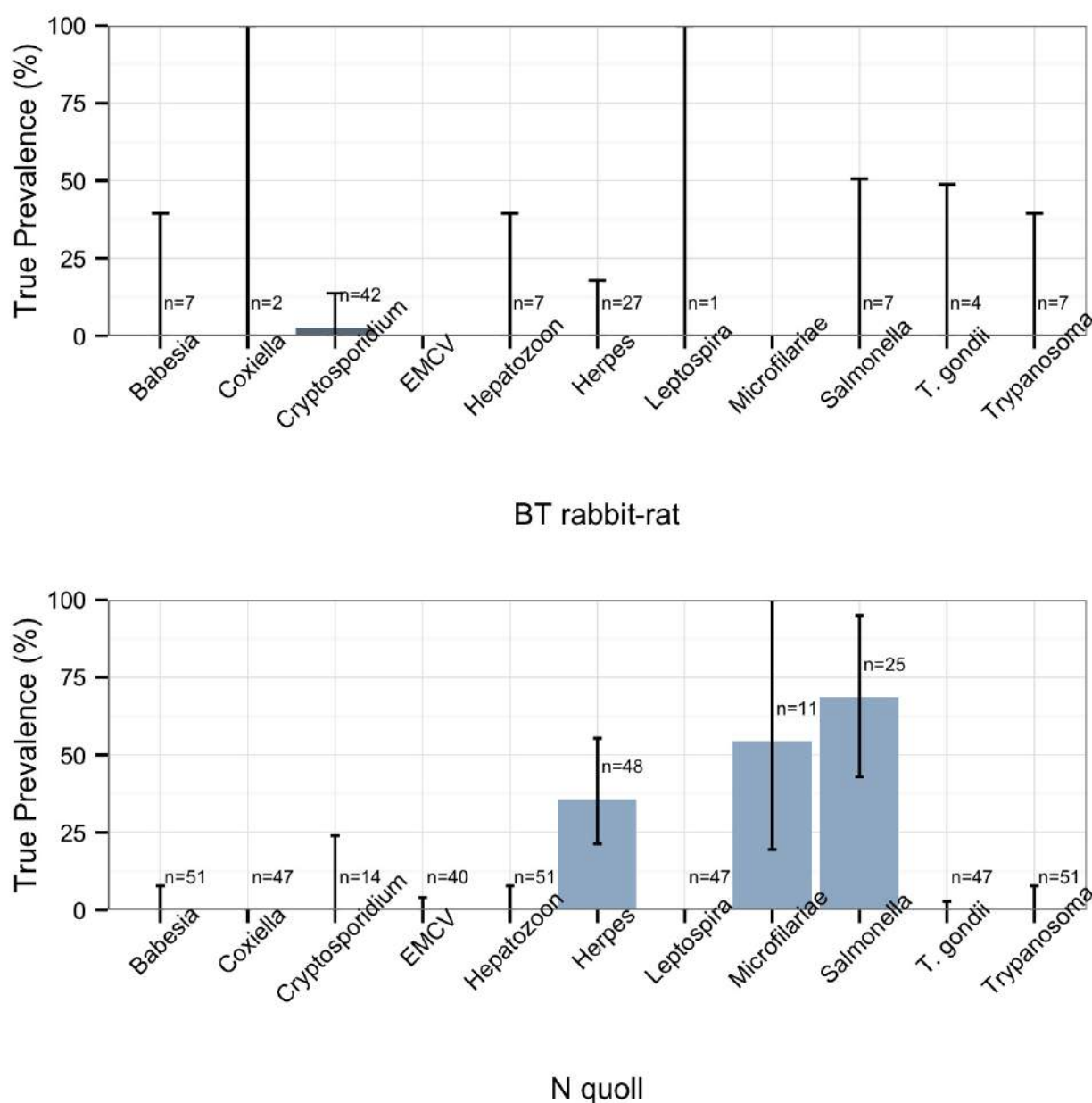


Figure 18: True prevalence results, by host, for all pathogens tested in target species.

### 3.8.1 Direct evidence of pathogen presence

#### 3.8.1.1 Herpesvirus infection

Herpesvirus PCR was performed on oral, conjunctival and rectal/cloacal samples representing 206 individuals with a true prevalence across all locations and species of 16.6% (95% CI: 11.4-23.9%), based on an assumed Se of 0.7 and Sp of 1.0 (Fig. 19).

Samples from individual swabs (cloaca, oropharynx and conjunctiva) were tested separately for all animals that returned positive results from pooled swab samples. HV were detected from all anatomical sites, however the oropharynx was the site which yielded most positive results. Preliminary data indicates all PCR positive animals were positive on their oropharyngeal swab. Few cloacal swabs (n=2) returned positive results.

All HV detected were gammaherpesviruses. Preliminary results of viral sequencing indicate that two different and potentially novel herpesviruses were detected. The HV found in northern brown bandicoots is most closely matched to asinine HV5. The HV found in northern quolls is most closely matched to *Sus barbatus* lymphotropic HV1.

There was no clinical evidence of disease associated with HV in any individual. Two animals from Kakadu National Park (an adult male northern brown bandicoot and a female northern quoll), had a change of HV status from negative to positive, when retested after several months, during the course of the investigation.

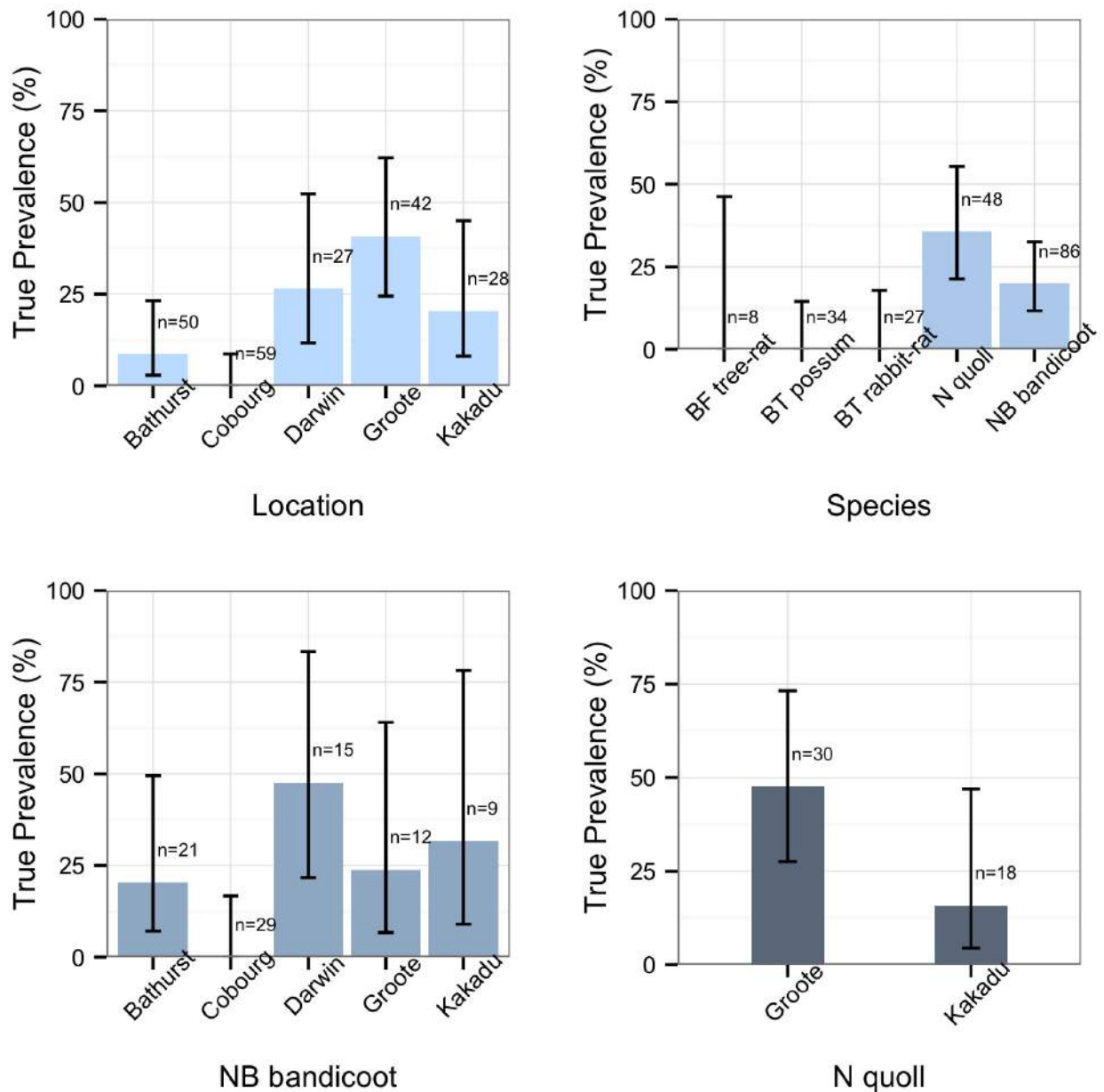


Figure 19: True prevalence for herpesvirus PCR of pooled oral, conjunctival and rectal/cloacal swabs across all locations and selected species, based on an assumed Se of 0.7 and Sp of 1.0.

A significant location effect was found for HV detection across all species when comparing Bathurst Island/Darwin/Groote Eylandt/Kakadu National Park against Cobourg, where no HV was detected (OR=23.6, 95% CI: 1.4-395,  $p=0.0002$ ). This location effect only remained for northern brown bandicoots when comparing the Darwin population with Cobourg (OR=20.9, 95% CI: 1.1-404,  $p=0.01$ ). No location effect by species was detected for northern quolls.

Across all species, within the islands HV detections were significantly higher on Groote Eylandt than on Bathurst Island (OR=6.3, 95% CI: 1.6-24.1,  $p=0.005$ ). Notably, there was no significant difference between HV detections on Groote Eylandt when compared to Darwin or Kakadu National Park, neither when comparing Bathurst Island with Darwin/Kakadu National Park. No seasonal differences were found for HV across the whole cohort, nor within northern brown bandicoots and northern quolls.

Packed cell volume was significantly lower in northern brown bandicoots with HV infection (mean=34.1) compared to those without (mean=37.6,  $p=0.04$ ). A significant sex association was also found across all species, with males more likely to be HV positive than females (OR=2.6, 95% CI: 1.0.-6.9,  $p=0.04$ ).

Herpesvirus was only detected in two non-rodent target species (northern quoll and northern brown bandicoot). This difference was significant when comparing the odds of HV detection in northern quolls versus all rodents combined (OR=26.4, 95% CI: 1.5-462,  $p=0.0009$ ), however was less significant between northern brown bandicoots and all rodents (OR=12.9, 95% CI: 0.7-224,  $p=0.02$ ).

### 3.8.1.2 *Salmonella* spp.

*Salmonella* cultures were performed on 86 individuals, with a true prevalence across all locations and species of 39.9% (95% CI: 27.9-54.5%). True prevalence estimates for *Salmonella* across locations and species were based on an assumed Se of 0.7 and Sp of 1.0 (Fig. 20).

No significant differences were detected for *Salmonella* test results between locations; between islands and the mainland; and between seasons (wet/dry). Northern brown bandicoots were significantly more likely to be *Salmonella* positive compared with brushtail possums ( $p=0.01$ ), as well as all rodents combined ( $p=0.02$ ). Likewise, the odds of being *Salmonella* positive was significantly higher in northern quolls versus brushtail possums (OR=28.7, 95% CI: 1.55-532,  $p<0.0001$ ), and northern quolls versus all rodents combined (OR=23.15, 95% CI: 1.24-433,  $p<0.0001$ ).

Within the positive species (northern quoll and northern brown bandicoot), there was no significant location effect detected for northern brown bandicoots, however there was a significant association between location and *Salmonella* detection in northern quolls for Groote Eylandt versus Kakadu National Park (OR=2.57, 95% CI: 1.25-5.28,  $p=0.03$ ).

There was no clinical evidence of disease associated with *Salmonella* in any of the 24 individuals which were positive for this pathogen. No significant associations were found for *Salmonella* detections when examined against PCV, TPP, Glob, Alb, body weight, TWCC, BCS, age class, sex or ectoparasite burden. However the odds of *Salmonella* detection were significantly higher for individuals with tooth wear grades 0 and 1, versus grades 2 and 3 (OR=6, 95% CI: 1.7-21,  $p=0.01$ ).



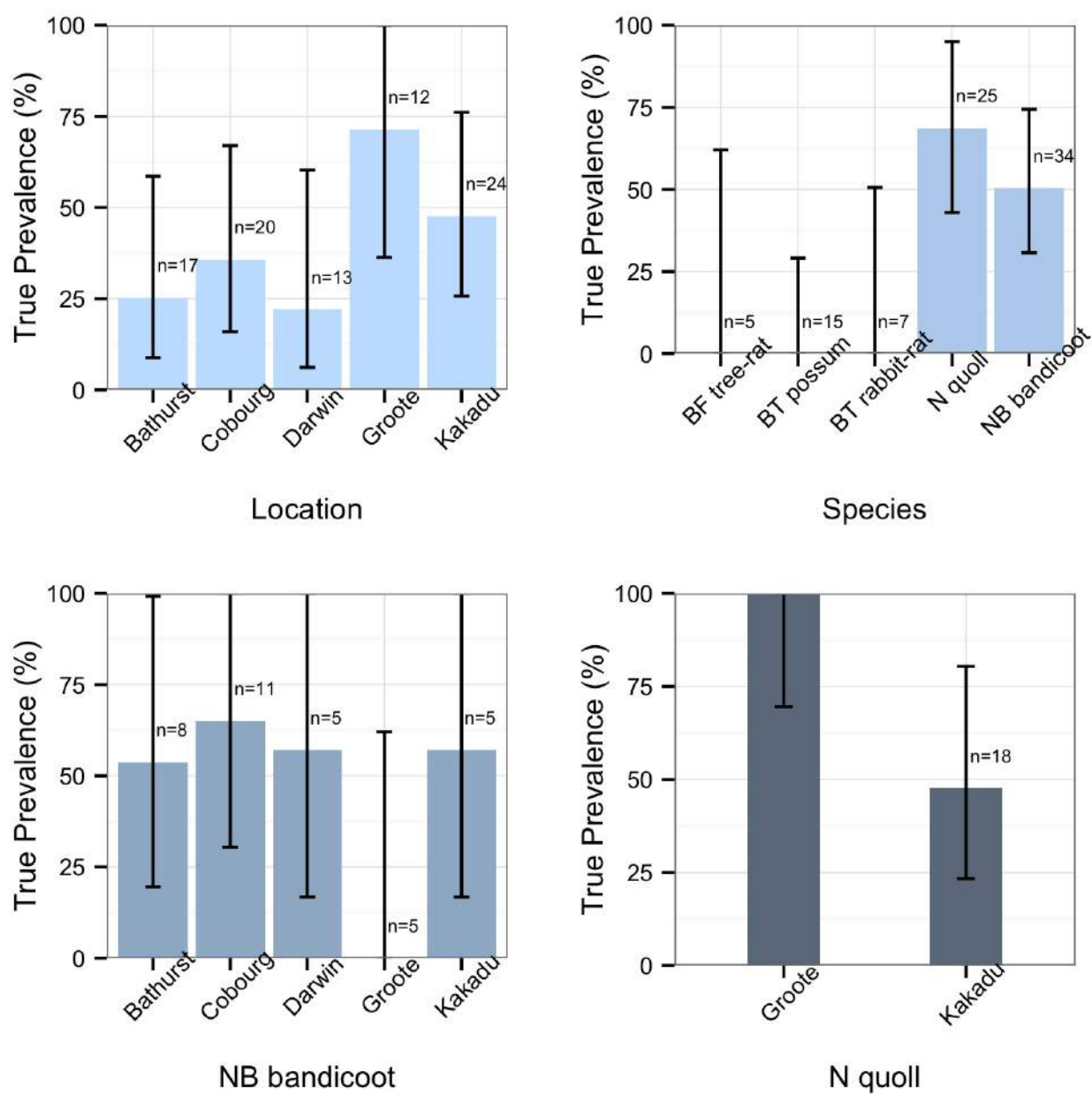


Figure 20: True prevalence for *Salmonella* culture across all locations and species, based on an assumed Se of 0.7 and Sp of 1.0.

A large number of *Salmonella* serotypes were cultured (Table 9).

Table 9: List of *Salmonella* serotypes cultured, numbers of isolations, locations and host species.

Serotype	No. of isolations	Locations	Species
<i>Salmonella</i> Aberdeen	1		NBB
<i>Salmonella</i> Adelaide	1	GRE	NQ
<i>Salmonella</i> Bahrenfeld	1	KNP	NQ
<i>Salmonella</i> Ball	3	COB, DAR	NBB
<i>Salmonella</i> Bronx	2	GRE, KNP	NBB, NQ
<i>Salmonella</i> Bukavu	1		NQ
<i>Salmonella</i> Cairns	1		NBB
<i>Salmonella</i> Chester	1		NBB
<i>Salmonella</i> Eastbourne	1		NQ
<i>Salmonella</i> Kisarawe	1	BAI, COB	NBB, NQ
<i>Salmonella</i> Ohlstedt	2	BAI, GRE	NBB, NQ
<i>Salmonella</i> Poona	1		NQ
<i>Salmonella</i> Rubislaw	1		NBB
<i>Salmonella</i> subsp. 1 ser 16:1,v:-	1	KNP	NQ
<i>Salmonella</i> subsp. 2 ser 21 : z10 : z6 (Wandsbek)	2	KNP	NQ
<i>Salmonella</i> Thompson	1		NBB
<i>Salmonella</i> Urbana	2	DAR, GRE	NBB, NQ
<i>Salmonella</i> Virchow PHAGE-TYPE: 8	1		NQ

### 3.8.1.3 Trypanosomes

Molecular investigation for blood borne protozoal parasites (*Trypanosoma* spp., *Babesia* spp. and *Hepatozoon* spp.) was performed on 209 individuals. Across all locations and species the true prevalence of *Trypanosoma* spp. was 21% (95% CI: 15.5-27.2%) based on an assumed Se of 0.9 and Sp of 1.0 (Fig. 21). Preliminary phylogenetic analysis indicated that the 30 trypanosome sequences isolated from northern brown bandicoots are most similar to *T. vegrandis*. The nine trypanosome isolates found in brushtail possums were most similar to *Trypanosoma* sp. AP-2011a isolate 64 from brushtail possums in WA. Six of 113 (5%) northern brown bandicoots were co-infected with *T. vegrandis* and *Hepatozoon* spp. and 8/113 (7%) northern brown bandicoots were co-infected with *T. vegrandis* and *Babesia* spp.

Northern brown bandicoots positive for trypanosomes had significantly lower PCV ( $p=0.046$ ) than negative northern brown bandicoots. Animals with lower tooth wear scores (0, 1) were more likely to be trypanosome positive than those with higher tooth wear scores (2, 3) (OR=2.74, 95% CI 1.3-5.7,  $p=0.006$ ). For both brushtail possums and northern brown bandicoots, no significant associations were found for trypanosome detections when examined against TPP, Glob, Alb, TWCC, BCS, age class or sex. The odds of animals being *Trypanosoma* spp. positive were significantly higher in 2013 versus 2014 (OR=3.97, 95% CI 1.79-8.82,  $p<0.0001$ ), however no seasonal association was found overall.

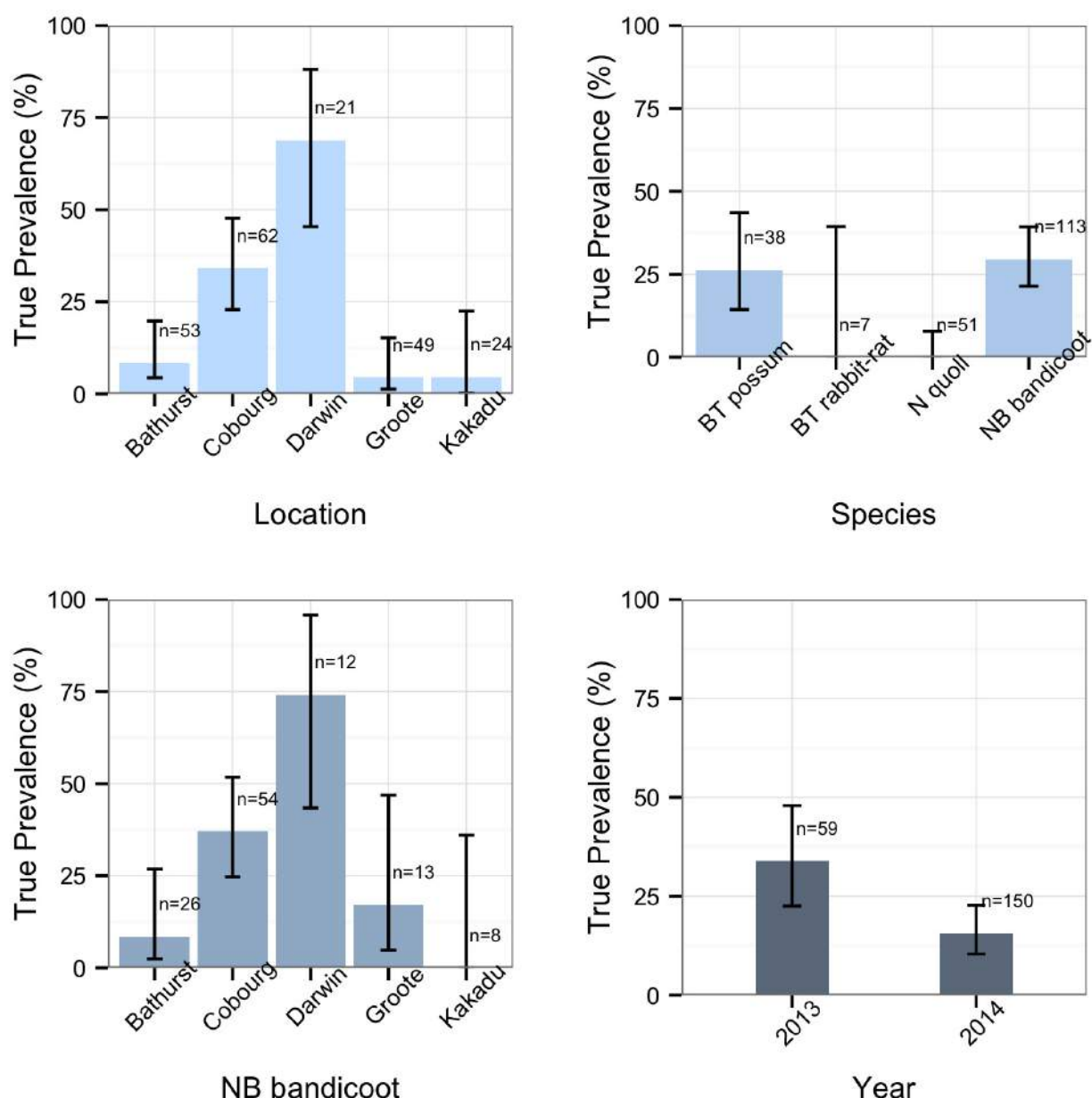


Figure 21: True prevalence for *Trypanosoma* spp. PCR of blood across all locations, species and years, based on an assumed Se of 0.9 and Sp of 1.0.

#### 3.8.1.4 *Babesia* spp. and *Hepatozoon* spp.

Across all locations and species, the true prevalence of *Babesia* spp. was 5.8% (95% CI: 3.3–10.2%) and of *Hepatozoon* spp. was 5.8% (95% CI: 3.3–10.2%). Assumptions were Se 0.9 and Sp 1.0 (as all positives reported had sequences obtained to verify the result).

*Babesia* spp. were detected in 0/38 brushtail possums, 0/7 brush-tailed rabbit-rats, 11/113 northern brown bandicoots and 0/51 northern quolls. Of positive northern brown bandicoots, 1 was from Bathurst Island, 7 from Cobourg Peninsula and 3 from Darwin. There was no clinical evidence of disease associated with *Babesia* spp. in any of the individuals which were positive for this pathogen.

*Hepatozoon* spp. were detected in 0/38 brushtail possums, 0/7 brush-tailed rabbit-rats, 11/113 northern brown bandicoots and 0/51 northern quolls (Fig. 22). Of positive northern brown bandicoots, 1 was from Bathurst Island, 5 from Cobourgh Peninsula, 2 from Darwin and 3 from Kakadu National Park. There was no clinical evidence of disease associated with *Hepatozoon* spp. in any of the individuals which were positive for this pathogen.

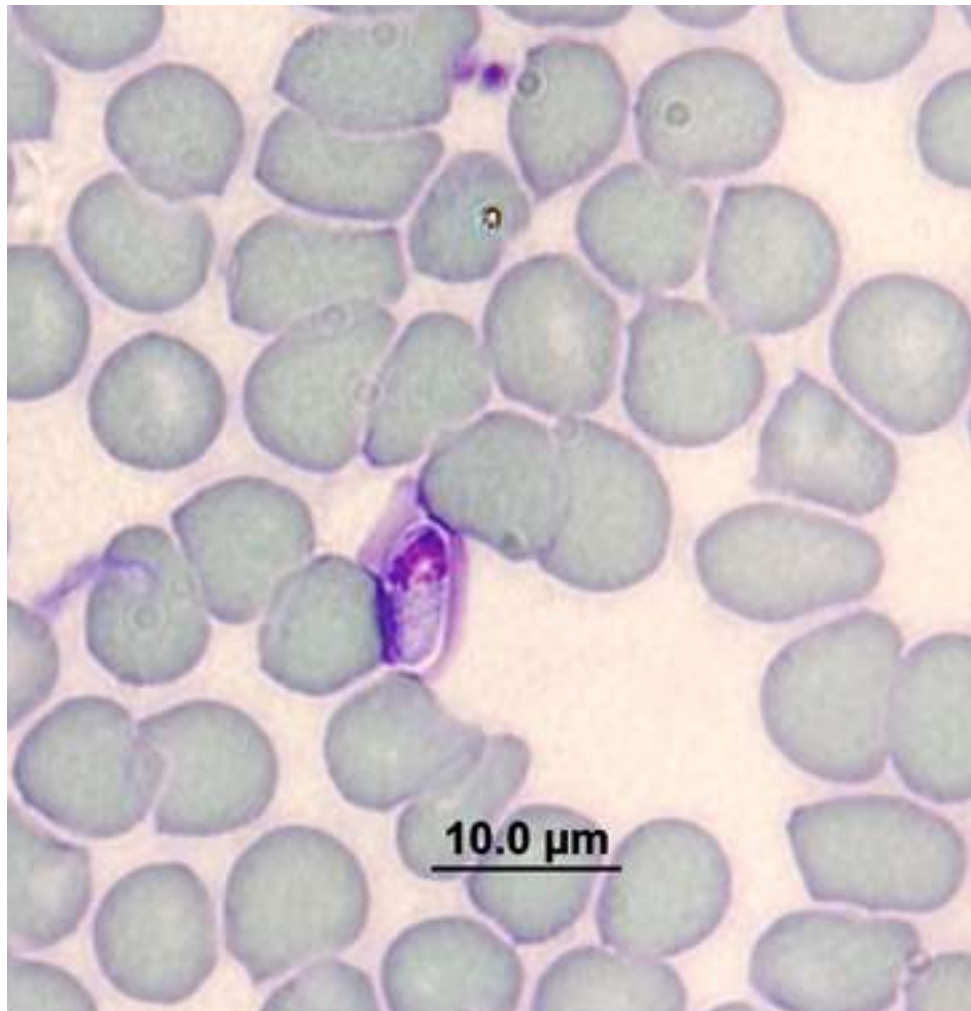


Figure 22: Photomicrograph of a *Hepatozoon* parasite within the red blood cell of a northern brown bandicoot from Kakadu National Park.

### 3.8.1.5 Microfilariae

Blood smears (n=77) were examined for microfilarial blood parasites. Assumptions were Se 0.5 and Sp 1.0 (as all microfilaria have a characteristic appearance and are unlikely to be confused with any other organism when blood smears are manually evaluated).

Across all species, there was a significantly higher likelihood of microfilaria detection on islands compared with the mainland (OR 11.1, 95% CI: 1.5-81,  $p=0.0016$ ) (Fig. 23). Brushtail possums were significantly more likely to have microfilaria than northern brown bandicoots (OR 12.8, 95% CI: 2.7-60,  $p<0.0001$ ). There were no significant differences in microfilaria prevalence in animals sampled in wet or dry seasons. No significant associations were found for microfilaria detections when examined against PCV, TPP, Glob, Alb, body weight, TWCC, BCS, age class, sex, tooth wear score or ectoparasite burden.

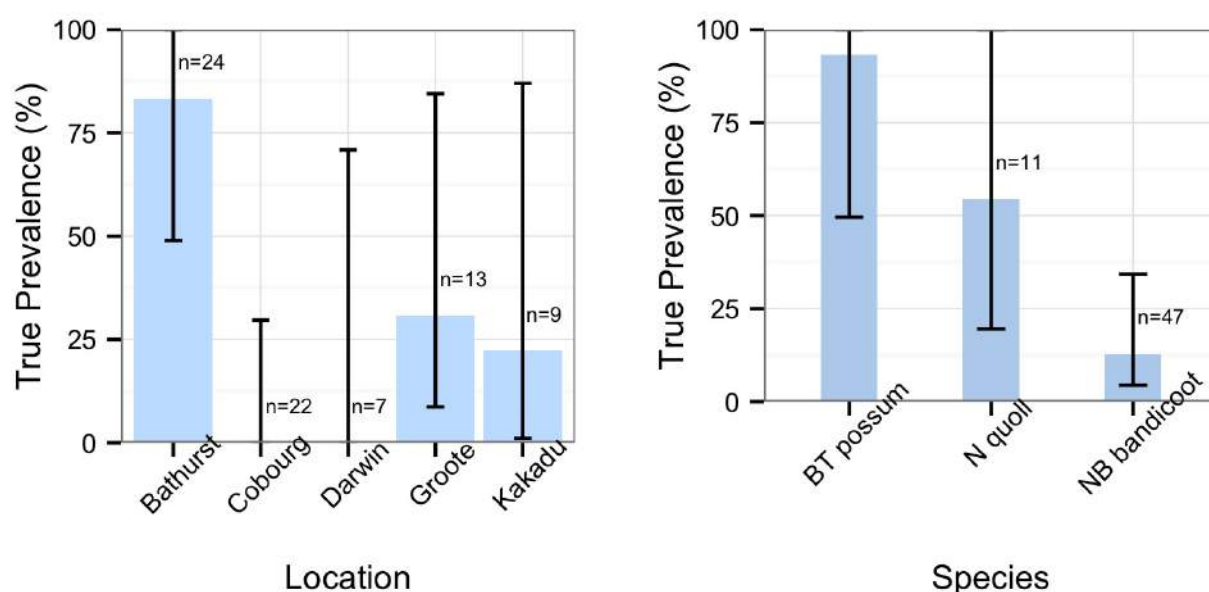


Figure 23: Test prevalence for microfilaria in blood smears across all locations and species, based on an assumed Se of 0.5 and Sp of 1.0.

### 3.8.1.6 Faecal samples

Microscopic examination of fixed faeces found no evidence of endoparasite burden (either helminth or protozoal) in a total of 110 faecal samples, including 2 black-footed tree rats, 33 brushtail possums, 23 brush-tailed rabbit-rats, 27 northern brown bandicoots and 23 northern quolls. This examination method for endoparasites was hampered to some extent by the small volume of faecal sample available in many cases.

PCR testing for the protozoal faecal pathogen *Giardia* spp. encountered some difficulties with molecular testing methodology. Preliminary evidence was found of positive samples in 20/147 (13.6%) animals across all species and locations, using quantitative PCR at the Gdh locus. Further results are pending.

PCR evidence of *Cryptosporidium* spp. was found in 1/5 *Antechinus bellus*, 0/7 black-footed tree rat, 1/56 brushtail possum, 1/42 brush-tailed rabbit-rats, 1 of 55 northern brown bandicoots, 0/14 northern quolls and 1 of 2 *Rattus tunneyi*. Positive animals were from Bathurst Island, Cobourg Peninsula and Kakadu National Park. *C. hominis* was detected in a northern brown bandicoot from Kakadu National Park, *C. parvum* from one *A. bellus* from Cobourg Peninsula, *Cryptosporidium* sp. isolate 21a from a brushtail possum at Bathurst Island and unidentified *Cryptosporidium* sp. from one *R. tunneyi* at Bathurst Island and one northern brown bandicoot at Cobourg Peninsula.

### 3.8.1.7 De novo molecular testing

*De novo* molecular testing was used to detect known and potentially unknown or novel pathogens. Testing of 38 northern brown bandicoot samples (pooled into four groups based on geographic location) revealed a variety of both DNA and RNA viruses with some differences evident between pools. The results to date are preliminary, with further analysis and interpretation pending. For any viruses that have potential significance as pathogens, more detailed bioinformatics and confirmatory molecular testing will be undertaken to better assess the identity and significance of these preliminary results.

Notable preliminary results include a strong representation of Retroviridae in samples from Bathurst Island, Cobourg Peninsula and Darwin, including the common possum retrovirus and a second retrovirus related to but distinct from koala retrovirus. It is possible that this is due to the presence of a single, novel retrovirus with sequences similar to both common possum retrovirus and koala retrovirus. Results will be prepared for publication.

### 3.8.2 Serological evidence of exposure to pathogens

#### 3.8.2.1 *Toxoplasma gondii*

Antibody testing for *Toxoplasma gondii* was performed on 205 individuals, with a true prevalence across all locations and species of 0% (95% CI: 0-0%), based on an assumed Se of 0.95 and Sp of 0.95 (Fig. 24). It should be noted the false positive rate from a Sp of 0.95 across the whole cohort accounts for an adjustment from an apparent (test) prevalence of 2% (95% CI: 0.8-4.9%) to a true prevalence of 0%.

Antibodies to *T. gondii* were only found in Darwin, with northern brown bandicoot the main species represented (3/4 positives in Darwin were northern brown bandicoots). Northern brown bandicoots were significantly more likely to be seropositive for *T. gondii* in the Darwin region compared with this species at Cobourg (OR: 34.26, 95% CI: 1.63-720,  $p=0.01$ ). Sample sizes in brushtail possums and northern quolls were sufficient to be confident the true seroprevalence for *T. gondii* in these species was either zero or very low, across all sites. Insufficient numbers of black-footed tree rats, black rats and brush-tailed rabbit-rats were sampled to be confident of the seroprevalence of *T. gondii* in these species.

Due to the small number of feral cats sampled ( $n=2$ ), with only one positive detected, it is not possible to infer a significant difference between seroprevalence for *T. gondii* in cats versus the native species tested. The sample size within northern brown bandicoot detections was too small for univariate analyses against PCV, TPP, Glob, Alb, body weight, sex, age class, ectoparasite burden or tooth wear. All three northern brown bandicoots positive for *T. gondii* were assessed to be in normal health on clinical exam, all had a BCS of 3 and one was co-infected with HV.



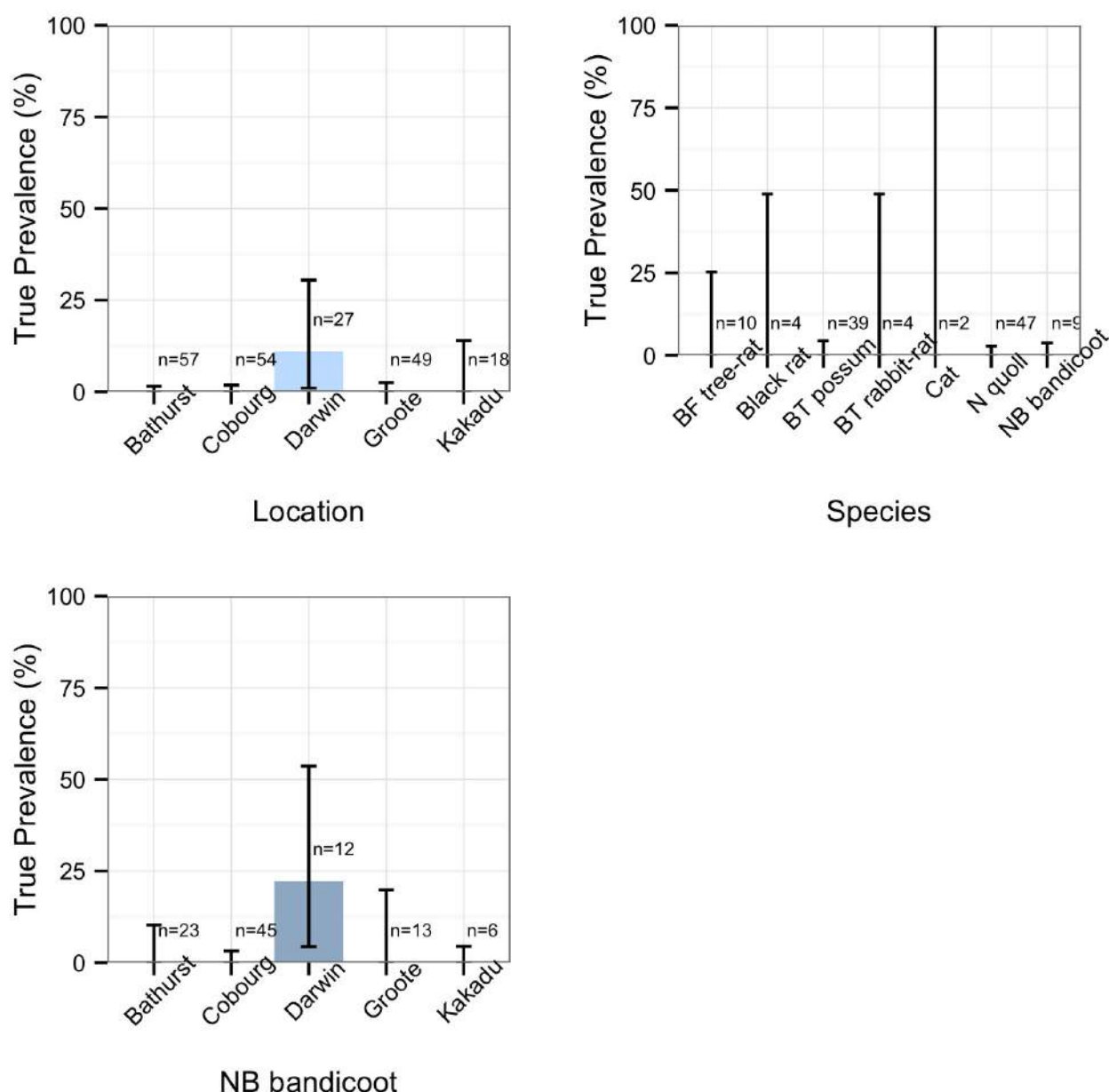


Figure 24: True prevalence for antibodies to *Toxoplasma gondii* across all locations and species, based on an assumed Se of 0.95 and Sp of 0.95.

### 3.8.2.2 *Leptospira* spp., *Coxiella burnetii* (Q fever) and EMCV.

There was no serological evidence of prior exposure to *Leptospira* spp. (n=168), *Coxiella burnetii* (Q fever) (n=183), or EMCV (n=148) across all species and locations tested (Figs. 25-27). For most of our study species and sites, sample sizes were sufficient to be confident the true prevalence was close to, or equal to 0%.



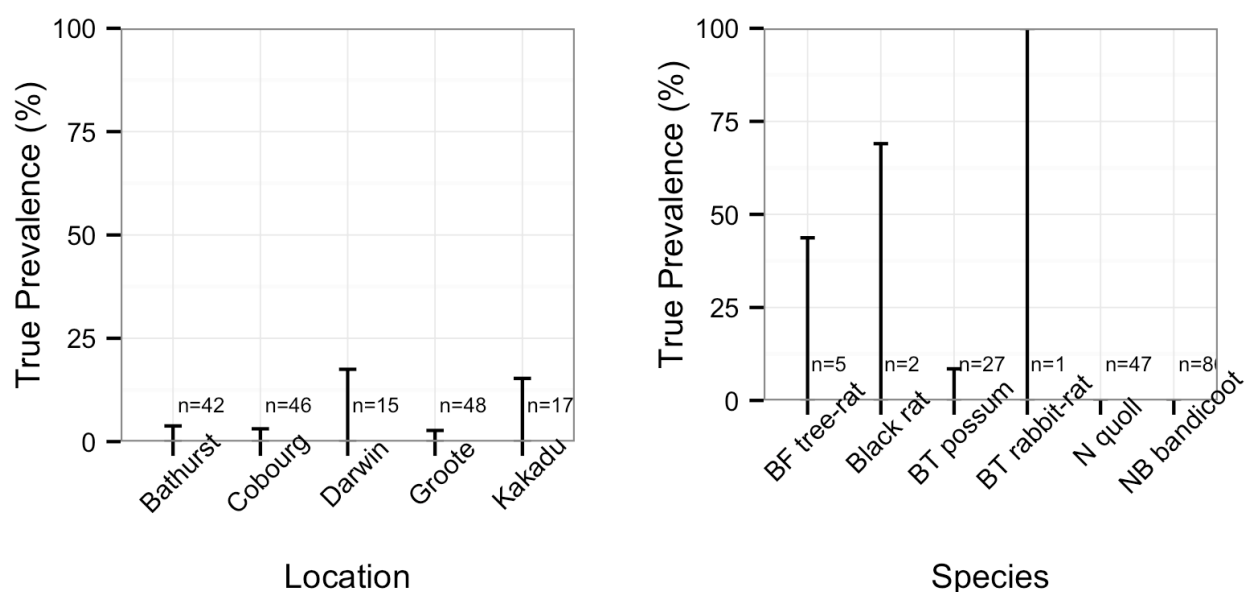


Figure 25: True prevalence for antibodies to *Leptospira* spp. across all locations and species, based on an assumed Se of 0.93 and Sp of 0.95.

Note all true prevalence estimates were 0%, with 95% confidence intervals indicating confidence of detection for species or location.

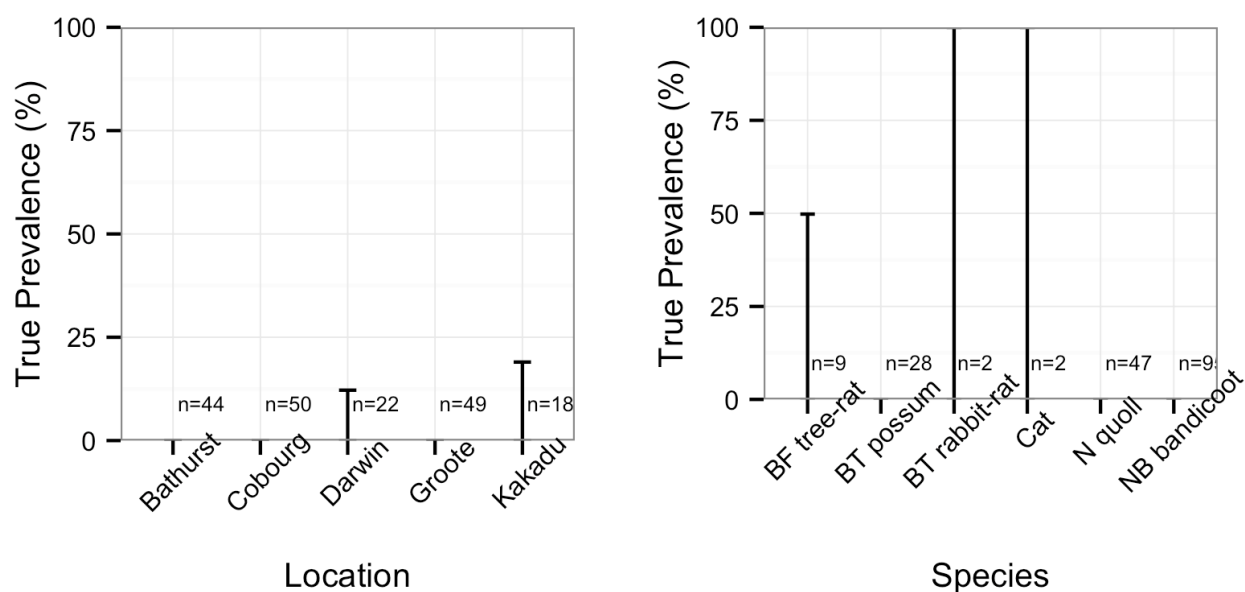


Figure 26: True prevalence for antibodies to *Coxiella burnetii* (Q fever) across all locations and species, based on an assumed Se of 0.5 and Sp of 0.9.

Note all true prevalence estimates were 0%, with 95% confidence intervals indicating confidence of detection for species or location based on sample size.

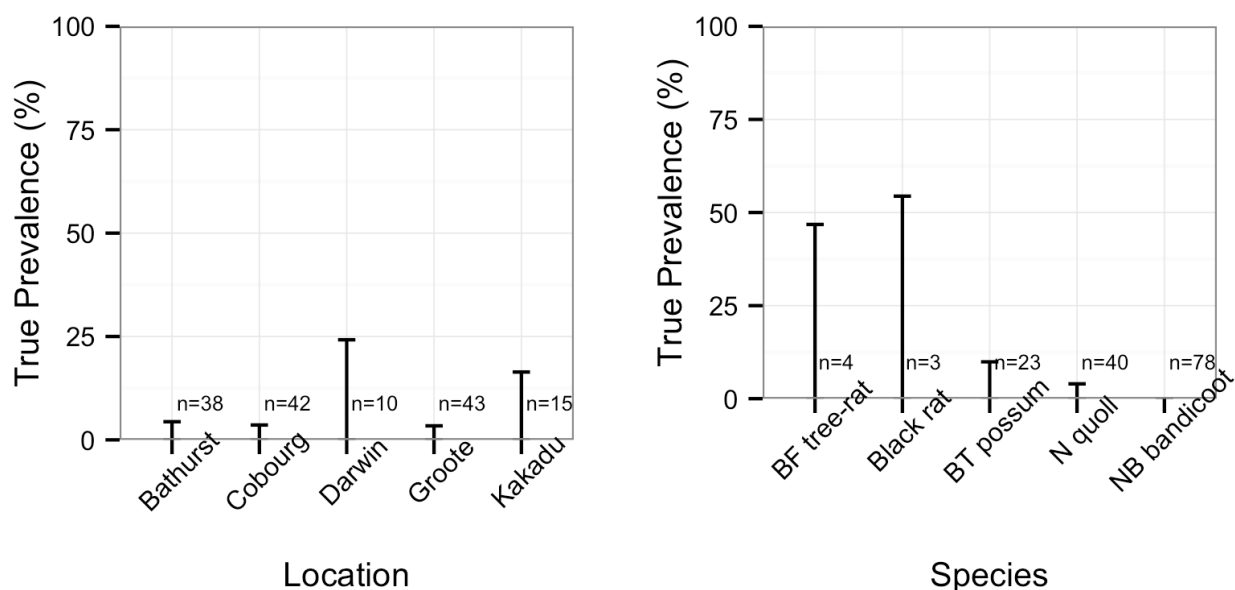


Figure 27: True prevalence for antibodies to Encephalomyocarditis virus across all locations and species, based on an assumed Se of 0.95 and Sp of 0.95. Note all true prevalence estimates were 0%, with 95% confidence intervals indicating confidence of detection for species or location based on sample size.

3.8.2.3 Ross River virus and Barmah Forest virus

Four of five black-footed tree rats, 2/2 brushtail possums, 3/12 northern brown bandicoots and 1/1 feral cat tested under a collaborative investigation undertaken by the NT Department of Health showed elevated antibody levels to RRV; 2 black rats were negative. There was no serological evidence of BFV in any animal tested: 5 black-footed tree rats, 2 black rats, 2 brushtail possums, 1 feral cat and 12 northern brown bandicoots (Kurucz *et al.*, in press).

3.8.3 Repeat sampling

One northern brown bandicoot and three northern quolls from Kakadu National Park were examined and sampled on more than two or three occasions, at least three months apart. Some repeat testing of these individuals was undertaken, with consistently negative serological results on each occasion for *T. gondii*, *C. burnetii*, *Leptospira* spp. and EMCV. However, the HV test status of two of these animals (an adult male northern brown bandicoot and a female northern quoll) changed from negative to positive when retested four, or six, months later.

3.9 Statistically significant variables

A summary of all statistically significant variables for pathogen outcomes using the Odds Ratio is presented in Table 10. Further detail on these results may be found under each pathogen’s section.

**Table 10: Summary of significant associations using the Odds Ratio for disease outcomes.**

Significance (p-values) were calculated using either uncorrected chi-square test (where all categories >5 samples) or two-tailed Fisher's exact test (any category <5 samples) as described in the methods; BTP=brushtail possum; NBB=northern brown bandicoot; NQ=northern quoll.

Pathogen	Association	OR	95% CI	p-value
<i>Salmonella</i> spp.	NBB vs. BTP	17.2	0.95-313	0.01
	NBB vs. all rodents	13.9	0.76-255	0.02
	NQ vs. BTP	28.7	1.6-532	<0.0001
	NQ vs. all rodents	23.2	1.2-433	<0.0001
	NQ (Groote) vs. NQ (Kakadu)	2.6	1.3-5.3	0.03
	All species tooth wear 0/1 vs. tooth wear 2/3	6	1.7-21	0.01
Herpesvirus	All other sites vs. Cobourg	23.6	1.4-395	0.0002
	Groote vs. Bathurst	6.3	1.6-24.1	0.005
	All species males versus females	2.6	1.0-6.9	0.04
	NQ vs. all rodents	26.4	1.5-462	0.0009
	NBB vs. all rodents	12.9	0.7-224	0.02
Trypanosomes	All species tooth wear 0/1 vs. tooth wear 2/3	2.7	1.3-5.7	0.006
	2013 vs. 2014	4.0	1.8-8.8	<0.0001
Microfilaria	Islands vs. mainland	11.1	1.5-81	0.002
	BTP vs. NBB	12.8	2.7-60	<0.0001
<i>Toxoplasma gondii</i>	NBB Darwin vs. NBB Cobourg	34.3	1.6-720	0.01

## 4 Discussion

This study greatly expanded understanding of disease in small mammal populations in the Top End, including the potential role of disease as a cause of population declines. Our investigation sampled nearly 400 individuals, from four target species and eight other species, across five major locations in the Top End. The samples and data collected during this project represent the largest set of wildlife health information collected in the Top End of the NT, and likely the largest data set of this kind in the tropical north of Australia. Our study is amongst a small number of projects worldwide to have approached investigation of disease in declining wildlife populations in this comprehensive and structured manner.

The population declines in the Top End have occurred across a wide range of mammal species and across at least four different taxonomic groupings, including both marsupials and eutherian mammals. It would be unusual for one infectious disease to impact such a range of species across several families and two orders (Pacioni *et al.*, 2015). There are few currently known pathogens that can affect such a broad taxonomic range sufficiently to cause widespread population declines, although chytrid fungus has had such an effect on anuran species (Skerratt *et al.*, 2007). Our hazard assessment identified *T. gondii* as the only known pathogen with the potential to exert population level effects on a broad range of mammals within a tropical ecosystem. The potential for Emerging Infectious Diseases and novel agents to exert effects in these populations is recognised and pathogen discovery work was undertaken, in part, in an attempt to address these concerns.

It was outside the scope of our study to investigate the spatial and temporal pattern of the current declines, however the existing information on small mammal declines in the Top End does not appear to be of sufficiently fine scale or power to be able to determine with any certainty whether the declines fit a pattern that could be attributed to the spread of a disease epidemic. Although our study did not specifically investigate non-infectious disease processes (e.g., toxins, nutritional deficiencies and genetic issues), many general health assessments undertaken in this investigation (clinical and haematological examinations, biochemistry profiles and skin biopsies) provide information on both infectious and non-infectious processes. The participation of experienced wildlife veterinarians and vet nurses with clinical expertise in the field surveys maximised opportunities for health assessment and biological sample collection, and ensured that subtle signs of ill health or disease could be detected.

## 4.1 Health assessments and demographics

The vast majority (94%) of animals examined under anaesthesia were considered to be in normal health, as judged by clinical examination, and the number of individuals in abnormal health is probably as expected in a 'healthy' free-ranging population. Both chronic and acute states of disease were found, in roughly equal proportion. Other than skin disease, no syndromes were consistently found in those individuals in poor health and the types of ill health described do not point to any significant patterns or syndromes of disease.

All wildlife disease studies are inherently biased and trapping for sampling introduces further biases. However it is difficult to know the direction in which these biases may affect the data, which will be dependent in part on the disease processes and the species in question (Nusser *et al.*, 2008). An abundance of trapped males compared to females is not unusual in small marsupial species as males often have larger home ranges and females are generally considered less likely to enter traps than males (due to greater timidity), although this may vary according to species and reproductive status (Statham and Statham, 1997, Hazel *et al.*, 2000, Oakwood, 2000, Pardon *et al.*, 2003, Richards and Short, 2003).

## 4.2 Body condition and weights

The findings of body condition in this study (81% of individuals with BCS 2.5 or higher; 16% BCS of 2) are similar to what one would expect to find in a healthy wild population, although our interpretation is subjective, due to the limited availability of baseline or reference ranges for our target species at these locations.

A location effect was found for very poor body condition, across all species; BCS 1 and 1.5 were significantly more likely at Bathurst Island than other sites. There may be factors in the environment (e.g., resource limitations or other stressors) that are contributing to this effect that our study did not reveal, and these factors may also be transient.

In general, northern quolls and male northern brown bandicoots at Kakadu National Park were significantly heavier than at other locations however the reasons for this effect are not clear. It may be a manifestation of the 'island rule', whereby members of a species get smaller or bigger depending on the resources available in the environment (Lomolino, 1985). Due to the apparent low density of small mammals at survey locations within Kakadu National Park (very low trap rates at Kakadu National Park compared to other sites) there may be greater availability of resources and less competition at this location. There may have been previous genetic selection for better muscled or higher conditioned individuals as a result of previous negative pressure on this population. As feral cat predation is

hypothesised to be exerting a significant effect on Kakadu National Park mammal populations, it is possible that smaller, less robust individuals were more commonly predated (being easier prey), resulting in a genetic selection in survivors for more robust body types or that smaller individuals are less available to be trapped. Alternatively, we also consider whether Kakadu National Park individuals are healthier than individuals from other populations, and that this is reflected in higher body weights. As morphometric measurements have not been analysed against weight to infer body size, our study found no other data to support this hypothesis.

### 4.3 Skin and ectoparasites

In our study, the vast majority of individuals with ectoparasites had only low burdens. A high prevalence of ectoparasites (albeit at low burdens) can be expected in wild populations, in particular in a tropical environment, where ticks are known to be a significant part of the biota (Jongejan and Uilenberg, 2004). The fact that only small numbers of individuals had moderate or high ectoparasite burdens supports the hypothesis that the levels of ectoparasitism seen are within what could be considered 'normal' in wild populations in such an environment.

The reporting of a new tick species in the NT (*Haemaphysalis novaeguinae*) and the first ever recognition of immature stages of this tick are important steps in improving and documenting our knowledge of ticks in the NT and throughout Australia. Given the role ectoparasites can play in disease transmission within wildlife populations, as well as in humans and domestic animals, documenting species of ectoparasites and host associations assists future public and wildlife health studies (Jongejan and Uilenberg, 2004).

As the skin is the most easily observable body organ, it can be expected that signs of disease, including the presence of ectoparasites, are more easily identifiable than with other organ systems. Skin disease was noted in a significant percentage of animals examined. Of the 17 individuals in our study assessed to be in abnormal health, skin disease formed a part, or a majority, of the issues noted for nine. We do not consider this unusual, especially in animals undergoing live trapping and in species where aggressive interactions are known to occur, such as in northern brown bandicoots and northern quolls (Stoddart and Braithwaite, 1979, Oakwood, 2000).

The further investigation of skin lesions through histopathological examination did not reveal issues of great concern at a population level. Papillomaviruses are often found in wildlife species and are generally opportunistic and host-species specific; their clinical expression may be associated with immunosuppression (Sundberg *et al.*, 2001). Wart-like lesions associated with a novel papillomavirus have been reported on the ventral tail of a brushtail possum in NZ (Perrott *et al.*, 2000). Poxvirus has also been reported in a brushtail possum (Samuel, 1989), but there appear to have been no published cases of papillomavirus in brushtail possums in Australia. The conservation significance of this possible papillomavirus is unclear at this stage but given clinical signs were detected in only one individual during this study it is unlikely to be of high concern. It is likely that the virus that caused the papilloma has been present in NT brushtail possum populations for many centuries and it probably does not pose a population threat. However poxvirus and papillomavirus have been reported to have population level threats on wildlife, including Australian mammals (Woolford *et al.*, 2007), so continued monitoring and investigation of such lesions is advisable.

Other histopathological skin investigation revealed chronic dermatitis secondary to mite infestation in northern brown bandicoots. This is not considered unusual in wild species such as northern brown bandicoots and one of the reasons for histopathological investigation was to rule out the presence of another underlying and unrecognised agent; no evidence of other agents was found. An increased prevalence of ectoparasite-related dermatitis in populations over time may be indicative of changes in ecosystems or in animals' immune-competence (Vogelnest and Woods, 2008).

Rump wear was detected in a relatively high percentage of brushtail possums examined at both Bathurst Island and Darwin. The detection of rump wear on Bathurst Island is not unexpected given the number of brushtail possums examined at this site, because rump wear is commonly reported in brushtail possum populations around Australia (Hufschmid *et al.*, 2010). Rump wear is a poorly understood disease of brushtail possums but is believed to occur more commonly in populations of high density and its presence and prevalence may be considered a useful indicator of the general 'fitness' of individuals (Hufschmid *et al.*, 2010).

Our study found no strongly significant, unexpected or novel findings when investigating skin disease and ectoparasites, although we describe several noteworthy cases of skin disease in individuals. We note that if vector-borne diseases are present or emerge in the Top End environment (in particular those transmitted by ticks) then transmission rates for such pathogens are likely to be high, due to the high level of ectoparasitism seen during our study.

## 4.4 Haematology and biochemistry variables

In our study, for northern brown bandicoots, PCV was significantly higher at Cobourg Peninsula and Kakadu National Park than Darwin; TPP at Bathurst Island was higher than at all other sites; and Alb was higher in Kakadu National Park than at Bathurst Island, Cobourg Peninsula and Groote Eylandt. The reasons for these differences are not clear although low PCV may provide an indication of individuals with blood loss, blood borne parasitism, chronic disease or malnutrition and altered TPP levels may indicate chronic disease or malnutrition (below normal) or dehydration, or acute infectious disease processes (above normal). Globulins measure the portion of plasma protein containing antibodies; elevation may be an indication of infectious processes. A low albumin may indicate malnutrition or chronic disease. An elevated TWCC can be indicative of an infectious or inflammatory process (Clark, 2004).

For brushtail possums, TPP at Darwin was significantly higher than Bathurst Island, which may be correlated to higher body weight for brushtail possums in Darwin compared to Bathurst Island (significantly so for male brushtail possums). This may indicate that brushtail possums at Darwin were in better body condition, and presumably better nourished than brushtail possums from Bathurst Island, and hence had a more 'normal' TPP. However, until reference ranges for haematological variables are calculated from our data, it is only possible to determine relative relationships for these variables (e.g., one group had higher or lower TPP) and we cannot speculate at this stage on which groups, if any, fall outside the normal range for these variables. Although there were no significant differences found between locations, within study species, for TWCC and Glob, sample sizes were small for these variables. Longitudinal studies with larger sample sizes, and inclusion of seasonal sampling, may reveal significant differences. Analysis of haematological and biochemical data gathered during this study has thus far been limited. More detailed analysis, including generation of suggested reference ranges for northern brown bandicoots (and potentially other target species), is planned and will be made available as a peer-reviewed publication. Large sample sizes are required to establish robust reference ranges for haematological variables for a species, and the effect of age, gender, physiological status (e.g., breeding) and season on these variables must also be considered (Clark, 2004).

## 4.5 Interpretation of pathogen tests

Interpretation of pathogen test results, both positive and negative, must be undertaken with care in a project such as this. Infectious agents are often a normal part of the biology of wild animals, and may be present without causing significant population level effects (Wobeser, 2006). When infection does result in disease in wildlife, outcomes may be difficult to assess, particularly impacts on survival and population size (Ryser-Degiorgis, 2013). Effects such as reduced fecundity, reduced longevity or increased risk of predation may be extremely difficult to determine unless studies are long term, focused and extensive in scope, including the use of mark-recapture studies for survival data (Wobeser, 2006). Highly virulent infections may kill affected animals and leave no easily detectable trace of their presence, if carcasses are lost to investigation. The potential for type II errors (failure to detect a significant association or difference) should be borne in mind for interpretation of results such as reported in this study, as this is a commonly encountered issue for decision-making in wildlife health studies (O'Brien *et al.*, 2009).

A lack of evidence for a pathogen (i.e. a negative test) does not imply that a pathogen is absent from a population, or that infectious disease is an insignificant factor in population declines. In general, highly virulent pathogens remove infected individuals soon after exposure, through rapid death or associated predation of diseased animals. The remaining population consists of unexposed individuals, with no serological evidence of exposure to the pathogen, and thus a low observed seroprevalence. On the other hand, if the disease effects of a pathogen are less severe, infected individuals will survive and remain to be detected during serosurveys. In this case the observed prevalence of the disease may be relatively high (Thrusfield, 2013).

As an example, if only a small number of individuals demonstrate serological evidence to Pathogen X, this may be interpreted in several ways. It may be an indication that:

- A significant number of individuals were infected with Pathogen X, but almost all died before they mounted an immune response, and thus were lost to investigation.
- Individuals infected with Pathogen X were sick, were sheltering and did not enter traps and hence were not tested.
- The samples for testing were not of sufficient quality for tests to yield positive results.
- The serological test (developed for domestic animals) was not able to detect antibodies in the wildlife species.
- The hosts had been infected in the past but antibody levels had fallen to undetectable levels by the time of sampling.
- Pathogen X is not present in the population and the low levels of positive tests are false positive results (as no test is 100% accurate).
- Infection rates with Pathogen X are low in the target population.



## 4.6 Direct evidence of pathogen presence

### 4.6.1 Herpesvirus

Our study detected HV in only two target species (northern brown bandicoots and northern quolls) and at four locations: Bathurst Island, Darwin, Groote Eylandt and Kakadu National Park, but not at Cobourg Peninsula. Herpesviruses may infect a range of wide range of mammals including Australian marsupials and rodents however the virus varies in host range and pathogenicity. All HV detected in our study were gammaherpesviruses which are generally highly host specific and are often not considered primary pathogens (Stalder, 2013). A gammaherpesvirus in macropods (Macropodid herpesvirus 3) was associated with disease and mortality in captive and free-ranging eastern grey kangaroos (*Macropus giganteus*) (Smith *et al.*, 2008b, Wilcox *et al.*, 2011). All HV can establish latent infections in various tissues of the body and reactivation of latent infection can occur, particularly in times of stress and immunocompromise (Roizman and Pellett, 2001).

Preliminary results of viral sequencing indicate that two different and potentially novel herpesviruses were detected, which is not unexpected as relatively little sequencing work has been undertaken on HV in Australian mammals and many mammal species have at least one host-adapted herpesvirus (J Devlin, personal communication, 2015). Further work will be undertaken to investigate these findings and their potential significance.

There was no clinical evidence of disease associated with HV in positive individuals and no significant associations between HV status and most physiological variables. Although PCV was significantly lower in HV positive northern brown bandicoots than in negative northern brown bandicoots, this effect was not repeated for brushtail possums or northern quolls, which may be due to small sample sizes. The lower PCV for HV positive northern brown bandicoots may be confounded by the finding that PCV for northern brown bandicoots at Cobourg Peninsula was higher than for northern brown bandicoots at Darwin and that there were no HV positive northern brown bandicoots at Cobourg Peninsula; thus this finding may be independent of HV status. It may also be reflective of an effect of HV infection on red cell production or loss in these individuals, or more likely, an indication that animals with lower PCV had reduced immune-competence and hence were more likely to be infected with, or be shedding HV. In this case the cause of the lowered PCV would be independent of the HV status of the individual. The cause of the lower PCV in HV positive northern brown bandicoots is not clear at this stage. Other than this finding, there were no other indications from our study that infection and shedding of HV was associated with expression of disease.

In our study, males of both affected species were more likely to be positive for HV than females and previous studies have shown that male marsupials are more likely to shed HV than females (J Devlin, personal communication, 2015). It is hypothesized that this may reflect a true difference in prevalence of infection between males and females and that behavioural difference may lead to greater opportunities for disease transmission in males. Alternatively, infected males may shed the virus more readily than females, for unknown reasons.

Interestingly, no HV was detected at Cobourg Peninsula (which was most significant for northern brown bandicoots; they were the target species most commonly sampled at Cobourg Peninsula, thus improving the power of this analysis). This could be indicative of a true absence of HV in all target species at Cobourg Peninsula or may reflect diminished viral shedding at this location, due to protective features of the environment which reduce the level of stressors acting upon individuals. For example, there may be a greater abundance of resources; reduced competition; and/or reduced predatory pressure at Cobourg Peninsula compared to other locations, which may manifest in animals shedding HV less frequently or at lower levels. If HV is truly absent from northern brown bandicoots

at Cobourg Peninsula, then a potential risk exists for this naïve population. If bandicoot HV were to be introduced to the Cobourg Peninsula population via, for example, trapping and handling procedures, translocation programs or unsanctioned release of hand-reared individuals, significant disease could potentially result.

Samples collected from the oropharynx were the most sensitive for detection of HV in our study. Our preliminary data indicates all PCR positive individuals were positive on their oropharyngeal swab and it may be easier and more cost-effective to only sample animals at this anatomical site in future.

Herpesvirus was not detected in rodents (three species,  $n=36$ ) nor in brushtail possums, which may be reflective of a true absence of HV in these species in the Top End or may indicate that infected animals were not shedding the virus when sampled. Additionally, the anatomical sites sampled in rodents may have been less than ideal for detecting HV. As rodents have no cloaca, the rectum was sampled, which would have only detected HV from the GIT, rather than a cloacal swab which could potentially detect HV from GIT, urinary and genital systems. Additionally, faecal contamination of rectal swabs may inhibit the sensitivity of the PCR (J Devlin, personal communication, 2015).

The change in HV status of two individuals from Kakadu National Park (an adult male northern brown bandicoot and a female northern quoll) from negative to positive during the course of the investigation is not unexpected. As mentioned previously, HV infections are often persistent but shedding is frequently intermittent. Additionally, test sensitivity will be dependent on the ability of the sampling procedure to collect the necessary quantity of viral DNA for a positive result. This finding emphasizes the potential lack of precision in any disease testing methodology and the fluid nature of disease states in individuals and populations. It also emphasizes the utility of mark-recapture studies, where animals are permanently identified and can be followed over time.

Despite recent studies in this area, understanding of HV in Australian native mammals and its potential role at a population level remains limited. Whilst our study detected HV only from clinically healthy animals, it is recognised that the virus may cause disease, both as result of novel exposure in naïve populations and reactivation of existing but latent infections (often associated with changing environments or increased stress) (Ladds, 2009, Vaz *et al.*, 2011, WHA, 2013a).

The potential clinical significance of HV infection in our target species and other species undergoing decline in the Top End remains unclear. The risk of novel HV infection in naïve hosts exists for Cobourg Peninsula and care should be taken to manage disease transmission risks, including appropriate hygiene measures during animal trapping and handling activities, and disease risk assessment and mitigation prior to any movement of wildlife. There is also risk of increased clinical disease associated with HV in all populations, under circumstances of increased stress, such as increased predatory pressure, reduced resources and climate change.

#### 4.6.2 *Salmonella* spp.

Although our study cultured *Salmonella* from almost 40% of animals tested, we found no clinical evidence of disease associated with infection. *Salmonella* has been frequently cultured from free-ranging native mammals in Australia, however in the majority of cases infection is generally considered not to cause disease (How *et al.*, 1982, Hart *et al.*, 1985, Ladds, 2009, WHA, 2009).

Animals infected with *Salmonella* may develop chronic carrier states and act as reservoirs for infection in other animals (Ladds, 2009, WHA, 2009). *Salmonella* prevalence may rise and infection may cause disease in individuals, including wildlife, when infective doses are high, when individuals are naive or have lower functional immunity and in situations of crowding, which may increase both infectious doses and levels of immune-compromise (CFSPH, 2005, Ladds, 2009).

In our study, individuals with lower tooth wear scores, and hence presumably younger in age, were more likely to be shedding *Salmonella*, perhaps because young animals had been recently exposed to infection and were shedding the bacteria at higher levels, or had yet to rid themselves of infection after initial exposure. This may be in part because younger animals may have lower levels of immune competence, or may be due in part to the nutritional effects mentioned below.

Seasonal variation has been reported, with higher *Salmonella* prevalence during the wet season in northern WA (How *et al.*, 1982) and during summer in temperate areas of WA (Hart *et al.*, 1985). The study by Hart *et al.* (1985) of quokka (*Setonix brachyurus*) on Rottnest Island (WA) found a relationship between population density, a lack of food resources over summer, and consequent rise in *Salmonella* prevalence from as low as 0% over winter to 100% in summer. The increased prevalence over summer is thought to be due to the seasonal shortage of carbohydrate in the quokka diet, leading to a rise in the pH of the stomach (Hart *et al.*, 1985). The low pH of the ruminant stomach is known to act as a defence against pathogenic organisms such as *Salmonella typhimurium* (Chambers and Lysons, 1979). Hart *et al.* (1985) concluded that *Salmonella* prevalence can be considered a useful indicator of environmental stress in the Rottnest Is. environment. Further focused studies of *Salmonella* shedding in the Top End may reveal similar resource and seasonal associations. We note the true prevalence in our study was already moderate to high (50 – 100%) in northern brown bandicoots and northern quolls, particularly on Groote Eylandt.

Enteric *Salmonella* may be shed intermittently by the host and ability to detect infection will be influenced in part by sampling methodology and ability to ship fresh, refrigerated samples to the laboratory in a timely fashion (OIE, 2008, WHA, 2009). In our study we were limited in our ability to transport fresh samples to the lab and some of our *Salmonella* samples were cultured after being frozen for weeks or months. These factors may have reduced our detection sensitivity and lowered the reported (apparent) and true prevalence of *Salmonella* in our study.

A variety of serovars were cultured from individuals in this study. All *Salmonella* serovars are considered potentially pathogenic, however many have a taxonomic or geographical predilection and baseline knowledge of presence and prevalence of *Salmonella* serovars in populations will aid in interpretation of findings if disease states emerge. All serovars are potentially zoonotic and *Salmonella* infection can cause serious GIT and systemic disease in humans (CFSPH, 2005, OIE, 2008). The zoonotic potential of infection should be considered whenever humans are interacting with wildlife, including indirect contact with potentially contaminated equipment, handling bags and traps (WHA, 2009). Appropriate Standard Operating Procedures covering hygiene and zoonotic disease risk minimisation should be followed by all staff working closely with wildlife.

Whilst there have been previous reports of *Salmonella* in brushtail possums from other parts of Australia and NZ (Presidente, 1984, Johnson and Hemsley, 2008), we failed to culture *Salmonella* from 15 brushtail possums tested. Likewise *Salmonella* was not detected in our study from brush-tailed rabbit-rats or black-footed tree rats. This may reflect a true absence of *Salmonella* infection in these species, or more likely, lower prevalence rates coupled with lower shedding, as it is known that different host species vary in how often and when they shed *Salmonella* and the amount of *Salmonella* shed (OIE, 2008).

*Salmonella* were cultured from northern brown bandicoots from all locations except Groote Eylandt, where only five individual northern brown bandicoots were tested. As *Salmonella* was cultured from all northern quolls tested at Groote Eylandt it is possible that *Salmonella* is also present in northern brown bandicoots on this island, although at lower prevalence. Northern quolls were significantly more likely to be *Salmonella* positive at Groote Eylandt than Kakadu National Park, although *Salmonella* was cultured from northern quolls at both locations. There may be a lower carriage of *Salmonella* in northern quolls at Kakadu National Park, or northern quolls could be shedding *Salmonella* at a higher level on Groote Eylandt. Although not likely to be significant from a health perspective, these findings may be indicative of differences in environment or host immunity, such as age class or species density.

The capacity for *Salmonella* to cause disease in wildlife under sub-optimal environmental situations is recognised and when *Salmonella* is present in wild populations, animals may be at increased risk when other diseases become active in the population (CFSPH, 2005, WHA, 2009). Additionally, an increase in *Salmonella* prevalence may serve as an indication of increasing environmental or host-related stresses (Hart *et al.*, 1985).

#### 4.6.3 *Babesia* spp. and *Hepatozoon* spp.

We found a low prevalence of *Babesia* spp. (10%) and *Hepatozoon* spp. (10%) in northern brown bandicoots only. These haemoprotozoa are rarely reported to cause disease in Australian wildlife, however *Babesia* has been associated with disease in brown *Antechinus* and eastern grey kangaroos (Cheal *et al.*, 1976, Barker *et al.*, 1978, Ladds, 2009) and *H. americanum* causes fatal disease in dogs in northern America (Chomel, 2011). The description of new *Babesia* spp. is not unexpected as relatively little sequencing work has been undertaken on *Babesia* in Australian mammal species and knowledge of these potential pathogens is limited. Our plans include analysis of *Babesia* and *Hepatozoon* prevalence against haematological reference ranges, once these have been developed.

#### 4.6.4 Trypanosomes

This study found a low overall prevalence of trypanosome infection in northern brown bandicoots and brushtail possums. Trypanosomes have been previously found in both these species but their presence has not been associated with clinical disease (Averis *et al.*, 2009, Thompson *et al.*, 2014).

Whilst our study reports first isolates of particular trypanosomes, this most likely reflects the limited study to date of trypanosomes in native species, including those in the NT. We found northern brown bandicoots with trypanosomes had lower PCV than negative northern brown bandicoots, which may be reflective of a direct effect of the haemoparasite on the host's red blood cells, or may indicate a less direct association with infection. Pathogenic trypanosome infections are closely associated with anaemia in other species, as a direct result of red cell parasitism, and infection may also be associated with immunosuppression (Onah *et al.*, 1998, Connor and Van den Bossche, 2004, Dargantes *et al.*, 2005). Animals with lower tooth wear scores (and presumably therefore younger) were also more likely to be trypanosome positive in our study, which may indicate parasite naivety or lower immune functionality in younger animals. The significant difference in *Trypanosoma* spp. prevalence in individuals sampled in 2013 against those sampled in 2014 is not apparently related to sampling during wet or dry seasons. The reason for this inter-annual variation is not clear but may reflect a larger cycle that is not obvious at this time or environmental or host differences between locations sampled in 2013 and those sampled in 2014. It is possible that fine-scale environmental variables between survey areas (such as proximity to fresh water sources or differences in vegetation) may favour vectors of trypanosomes at some sites over others. Little is known about vectors of trypanosomes in Australia, which limits opportunity to interpret this data further.

The lack of evidence of trypanosomes in rodents in our study may be reflective of the very low number of blood samples available for analysis. Thompson *et al.* (2014) drew attention to the relative paucity of information on trypanosomes in native rodents. More information on this host taxon is necessary for biodiversity conservation purposes and also to more accurately assess the potential risks of exotic trypanosomes making incursion onto the Australian continent. Exotic trypanosomes are most likely to gain entry to Australia along our northern coastline, which is in close geographical proximity to countries to our north known to host exotic and pathogenic trypanosomes such as *T. evansi* (the causative agent of Surra, a serious zoonotic disease) (Reid *et al.*, 2001, Thompson *et al.*, 2014). The Top End also possesses a suitable climate and likely vectors for transmission and continuation of these diseases. Experimental infection of Australian macropods with *T. evansi* resulted in high mortalities, indicating that all macropods, and potentially all Australian marsupial populations, could be highly vulnerable to Surra if exposed (Reid *et al.*, 2001). Such exotic trypanosomes have the potential to cause significant population level disease in Australia, in domestic animals and humans, as well as in wildlife populations (Desquesnes *et al.*, 2013).

The potential for trypanosomes to cause disease in Australian mammals is now recognised and recent studies have suggested trypanosome infection was associated with the decline of the woylie (Botero *et al.*, 2013); possible disease in koalas, including anaemia (McInnes *et al.*, 2011); and the historical extinction of MacLear's rat (Wyatt *et al.*, 2008, Thompson *et al.*, 2014). Whilst the significance of the trypanosomes found in our study remains unclear, we note that the risk of disease increases if new pathogens are introduced into naïve hosts.

#### 4.6.5 Protozoal endoparasites

Although our study found preliminary evidence of *Giardia* spp. infection in target species there was no associated evidence of disease; however, further analysis will be undertaken when more detailed results are available. The genus is considered a potential pathogen for all mammal hosts and infection in free-ranging wildlife may have impacts on domestic animal and human health (Bettiol *et al.*, 1997, CFSPH, 2012). Previous studies have shown low to medium prevalence of *Giardia* spp. infection in Australian marsupials (Thompson *et al.*, 2010a, Paparini *et al.*, 2010, Bettiol *et al.*, 1997). Further information and improved understanding of presence, prevalence and species of *Giardia* spp. in Top End small mammal populations would be beneficial for domestic, human and wildlife health efforts.

In our study, a variety of *Cryptosporidium* spp. was found in the faeces of a small number of individuals. The significance of *Cryptosporidium* spp. infections to populations in our study is unknown but there is no evidence to suggest that it is causing primary disease. *Cryptosporidium* spp. have previously been reported in rodents, bandicoots and brushtail possums (Ladds, 2009, Ryan and Power, 2012). In immunosuppressed hosts infection is known to cause disease and occasional fatalities (Ladds, 2009, Ryan and Power, 2012). The apparently novel species from Bathurst Island and Cobourgh Peninsula may be significant as, similar to *Giardia* spp., *Cryptosporidium* spp. infection in free-ranging wildlife may also have impacts on domestic animal and human health (Appelbee *et al.*, 2005). Further information and improved understanding of presence, prevalence and species of *Cryptosporidium* spp. in Top End small mammal populations would be beneficial to domestic, human and wildlife health efforts.



#### 4.6.6 Microfilaria

Microfilariae were significantly more likely to be found in brushtail possums than northern brown bandicoots and were also significantly more likely in island than mainland populations. These results may have been influenced by the larger numbers of brushtail possums and northern quolls (with higher prevalence of microfilaria) sampled at island locations and the larger number of northern brown bandicoots (with low microfilaria prevalence) sampled at mainland locations, or may be indicative of a true effect of geography or location.

Most filaroid nematode species are known only to parasitise a fairly narrow range of host species, so it is likely that microfilaria found in brushtail possums, northern brown bandicoots and northern brown bandicoots represent different species of filarial nematodes (Clark, 2004). Identification of the likely species of nematode would be useful and would allow greater interpretation of the significance of our findings, however this is a specialised skill (Clark, 2004). At this stage the significance of circulating microfilariae in our target species is not known, but it is possible that some are from species that have previously been associated with disease.

#### 4.6.7 Helminth endoparasites

Our testing for helminth endoparasites via faecal microscopy showed no positives, from 110 samples, and no evidence of GIT protozoa was detected via this testing method. The methodology was hampered to an extent by the small volume of faecal sample available from many individuals. Additionally, due to logistical constraints, testing was undertaken on ethanol-fixed samples. Greater information may have been obtained from fresh samples.

In general, we would expect a proportion of free-living small mammal populations to be harbouring GIT helminths, with burdens likely to be low (Vogelnest and Woods, 2008, Ladds, 2009). In our study, it is likely that at least some individuals (an unquantifiable proportion) in each of the target species would have had GIT helminth burdens, however, the numbers of helminth eggs and larvae passed in faecal samples was probably below the detectable levels for the testing methodology employed in this study.

The likely impact of any GIT helminths, if present, was not able to be quantified at either an individual or population level. We note, however, that the majority of GIT helminth species found in Australian native mammals are considered non- or lowly-pathogenic (Ladds, 2009). From the limited information gathered from this testing, we may reasonably conclude that, in general, GIT helminth burdens in our target populations are low or absent and are not primarily drivers of ill health.

We note that our testing methods for helminths were not able to gather information on potential helminth parasites outside the GIT (e.g., lung worms). Post mortem examination is generally required for identification of these types of helminths, as eggs and larvae are not shed in host faeces. Detection of microfilaria in the blood stream may provide information on some species of filaroid nematodes of the family Onchocercidae [see above] (Clark, 2004).

In general terms faecal parasitology (microscopic identification of parasite eggs and larvae in host faeces) has limited utility when attempting to identify parasites beyond broad taxonomic grouping. Even when eggs or larvae are identified in faeces, the specific type of parasite usually cannot be determined. Specialised techniques, requiring fresh faecal samples or post mortem examination of the GIT by specialised parasitologists are required for this. Additionally, it is impossible to infer likely disease effects from the results of a faecal parasite exam alone (Spratt *et al.*, 2008).



#### 4.6.8 *De novo* molecular testing

*De novo* molecular testing of samples from northern brown bandicoots revealed a variety of both DNA and RNA viruses, with some differences evident between testing pools. Many of the viral agents detected are likely to be from dietary sources, particularly when few aligned contigs (DNA segments) are detected. Putative agents with a low number of aligned contigs may be misclassified, as the detected contigs may be highly conserved among different viruses and align to the closest conserved region on GenBank. More detailed bioinformatics (including BLASTx analysis of all contigs of interest and confirmatory molecular testing) will be undertaken for any viruses of potential pathological significance and this will help to clarify the significance of these preliminary results. We note that our study swabs were collected from the rectal or cloacal mucosa, thus only organisms from those anatomical sites could be detected.

The techniques used in this study demonstrate the potential power of this tool when investigating disease in wildlife populations where baseline knowledge is limited. Preliminary results from *de novo* pathogen discovery work should be interpreted with caution until more information is available. A more detailed report and discussion, with recommendations, will be prepared for peer-reviewed publication once results are finalised and analysis and interpretation has been undertaken.

### 4.7 Serological evidence of exposure to pathogens

#### 4.7.1 Barmah Forest virus and Ross River virus

The investigation undertaken by the NT Department of Health in cooperation with the Department of Primary Industry and Fisheries and DLRM, on samples collected during our study revealed no serological evidence of BFV infection but serological evidence of infection with RRV in black-footed tree rats, brushtail possums, northern brown bandicoots and a feral cat in Darwin (Kurucz *et al.*, in press). RRV has not been associated with clinical disease in Australian native wildlife (Harley *et al.*, 2001, Ladds, 2009) and we did not find any evidence of clinical disease associated with this virus in our study. Neither pathogen is considered a primary concern for the health of Top End mammal populations. The information gained through this collaborative study will help to improve understanding of the epidemiology of these pathogens in the Top End, including that of potential reservoir or amplifying hosts.

#### 4.7.2 Encephalomyocarditis virus

Our studies suggest that EMCV was most likely absent from Top End small mammal populations at the time of this study as we found no serological evidence of EMCV in any animal tested, with a sufficiently large sample size across most species and locations. Rodents are thought to be the reservoir species for EMCV although the epidemiology is poorly understood and a role for feral pigs as reservoir species has also been proposed (Carocci and Bakkali-Kassimi, 2012). As no tests for EMCV were conducted on one of our target rodent species, brush-tailed rabbit-rats, due to difficulties in collecting serum samples, and only small numbers of other rodent species were tested, our study gained limited information on the presence of the virus in local rodents. Our results support earlier work by Jackson *et al.* (2010), where no evidence of serological response to EMCV was found in a study of black rats in the Top End.

In most mammalian species, EMCV infection results in sudden death (Thomson *et al.*, 2001). As with other highly virulent pathogens, after infection there may be few or no surviving individuals with detectable antibodies. Therefore the absence of antibodies to EMCV in our study may indicate an absence of the pathogen in the environment; that there is an as yet unknown and untested reservoir species; or that the pathogen is present but that no infected animals survived to demonstrate the presence of antibodies. Whilst we consider the last alternative less likely, such a possibility should be considered, particularly with an agent such as EMCV that is known to have a high fatality rate. Information gathered from dead or dying animals could provide further understanding and the value of studying animals within predator-proof areas is further highlighted.

EMCV is considered a zoonosis and in some areas of the world may cause significant human disease, although this does not appear to be the case in Australia (Kirkland *et al.*, 1989, Thomson *et al.*, 2001). Nevertheless, increased understanding of the epidemiology of EMCV in both animals and humans in Australia would be beneficial. It would also be useful to investigate the potential role of feral pigs as reservoirs of EMCV in the NT.

### 4.7.3 *Coxiella burnetii* (Q fever)

The absence of serological evidence of *C. burnetii* infection in our study is somewhat unexpected, given the confirmed presence of the causative agent in the Top End and the size of our sample. The incidence of disease in humans in the NT appears to be increasing, and in many cases a livestock route for transmission has not been found, suggesting that humans may be acquiring infection via other routes, including exposure to wildlife (Schultz *et al.*, 2007).

Previous studies in northern Qld have found evidence, by both PCR and ELISA serology, that around 30% of northern brown bandicoots were infected with *C. burnetii*. Brushtail possums were shown to be infected at lower prevalence and only by PCR (Cooper *et al.*, 2013). Studies undertaken by Bennett *et al.* (2011) found molecular evidence of *C. burnetii* in free-ranging western barred bandicoots and associated *H. humerosa* ticks, as well as serological evidence, by ELISA, of exposure in the host. An overall seroprevalence of 20.8% was found in macropods in Qld and WA, using ELISA, with a significantly higher prevalence in northern Qld compared to southern and western parts of that state (Cooper *et al.*, 2012).

Our results suggest the pathogen was circulating at zero or low prevalence across all locations at the time of our study, although we note that samples sizes in rodents were too small to draw meaningful conclusions in these species. It is possible that the lack of positive serological result in our study is a result of known limitations in serological testing and interpretation for *C. burnetii*. Antibody responses to *C. burnetii* are highly variable in humans and domestic animals and the same has also been shown to be true for wildlife exposed to *C. burnetii*, where studies showed that some individuals infected with *C. burnetii* develop long-lasting antibody response while other individuals develop no detectable response (Rousset *et al.*, 2004, Cooper *et al.*, 2013). Additionally, whilst the majority of exposed livestock remain seropositive for a number of years following acute infection (CFSPH, 2007), it is unknown for how long antibodies persist in Australian wildlife (WHA, 2013b).

Our technique for measuring antibody response to *C. burnetii*, the complement fixation test (CFT) (Ellis and Barton, 2003), was different to other recent studies in Australian wildlife, which used the ELISA test. Because there was very little information available on the use of this test in marsupials, we estimated the test sensitivity to be 50%. We chose the CFT because it was the only serological test commercially available to us in Australia and we had no access to a research lab running the ELISA test. Additionally, the CFT was run at the same commercial veterinary lab where *T. gondii* serology was performed, and both *C. burnetii* and *T. gondii* serology could be run on 0.1 ml of serum, which was of great benefit in our study where the volume of serum from individuals was limited. Nevertheless, the differences in testing methodology are noted, and we acknowledge that this makes comparison of findings and interpretation of results more challenging.

If *C. burnetii* was circulating in our target species at a prevalence found in other studies (e.g. >20%) and if the serological test we used was as sensitive and specific as we have estimated, we should have detected evidence of serological exposure to this pathogen, which we did not. Improved understanding of the presence, prevalence and impact of *C. burnetii* in Top End small mammal populations may be obtained if investigations are undertaken to look for molecular evidence of the pathogen in mammals and their ticks, using PCR, allied with serological assays using ELISA, where possible. Because of its high infectivity, wide host range and significance as a zoonosis, an improved understanding of *C. burnetii* epidemiology in the NT environment would be beneficial for domestic animal, wildlife and human health. At this stage, we cannot comment further on whether *C. burnetii* is exerting a population level effect on our target populations, but given the information available to date, this appears unlikely.

#### 4.7.4 *Leptospira* spp.

The absence of seropositivity to leptospiral infection in our study was unexpected, given our sample size and the expected seroprevalence for this pathogen when present in Australian mammal populations (Durfee and Presidente, 1979b, Milner *et al.*, 1981, Eymann *et al.*, 2007). *Leptospira* is known to be present in the Top End and clinical disease is seen in dogs in Darwin; however the maintenance host for these infections is not known (C Shilton, person communication, 2015). In northern Qld serovar Australis (the most common serovar in the Top End) has been associated with a carrier status in rats and bandicoots (CCRRL, 2010) and evidence of leptospiral infection has been found in brushtail possums, northern brown bandicoots, native rodents and dasyurids in other parts of Australia and NZ (Sullivan, 1974, Hathaway *et al.*, 1978, Durfee and Presidente, 1979a, Durfee and Presidente, 1979b, Hathaway, 1981, Milner *et al.*, 1981, Slack *et al.*, 2006, Eymann *et al.*, 2007, Smythe *et al.*, 2007). Reported seroprevalence from studies of brushtail possums in Australia and NZ ranges from 10-80% (Hathaway *et al.*, 1978, Durfee and Presidente, 1979c, Milner *et al.*, 1981, Day *et al.*, 1998, Caley and Ramsey, 2001, Eymann *et al.*, 2007) however there is comparatively little information available on leptospiral infection and expected seroprevalence in bandicoots and dasyurids. Our study had statistical confidence of detection if prevalence was above 10% in all target species, except the brush-tailed rabbit-rat.

Reported epidemiological features of leptospiral transmission and infection in Australian native wildlife include population density (Hathaway *et al.*, 1978, Day *et al.*, 1998), increasing age (Hathaway *et al.*, 1978, Eymann *et al.*, 2007) and seasonality. In tropical areas the seroprevalence of leptospiral infection increases during the wet season, as the agent survives longer in the environment during warm, moist conditions (Wobeser *et al.*, 2001, Centre for Disease Control, 2012). Most of our sampling occurred well into the dry season and it is possible that sampling during the wet season, or very early dry season, may have revealed a higher seroprevalence.

Rodents are known to act as maintenance hosts for *Leptospira* serovars and may also suffer disease as a result of 'accidental' infection with other serovars (Wobeser *et al.*, 2001). In our study, our small rodent sample size provides limited information on the presence of the bacterium in local rodents, but supports earlier work by Jackson *et al.* (2010), where no serological evidence of exposure to *Leptospira* spp. was found in a study of black rats in the Top End of the NT.

It is not known how long antibodies to *Leptospira* persist in wildlife hosts after infection; however it is reported that the antibodies detected in domestic species may persist for months or even years, although this may vary significantly between individuals. Eymann *et al.* (2007) concluded that antibodies to *Leptospira* persisted for at least a year in brushtail possums. However in experimentally infected brushtail possums, antibodies to infection had declined to low levels by day 40 post infection (Durfee and Presidente, 1979a).

Although we found no evidence that any of our target species were maintenance host species for *Leptospira* serovars detectable by our serological methods, it is possible that other unidentified serovars of *Leptospira* are circulating in Top End small mammals, undetectable by the current serovar panel testing. This would be unlikely given the current high level of knowledge of leptospires in Australia. If indeed our target species are not maintenance host species for *Leptospira* serovar Australis, then an improved understanding of other potential maintenance hosts for this bacterium in the Top End would be beneficial to domestic, human and wildlife health efforts. Leptospires could be a risk in Top End small mammal populations, particularly if populations are exposed to novel serovars. In these circumstances, as was shown by Durfee and Presidente (1979a) infection can result in clinically significant disease.

#### 4.7.5 *Toxoplasma gondii*

Our study revealed lower than anticipated levels of seropositivity to *Toxoplasma* in all our study species, and in particular in our three marsupial species, given the confirmed presence of feral cats at all study locations. Bandicoots appear to be highly susceptible to infection with *Toxoplasma*, most likely influenced by their behaviours of digging in, and feeding from, soil and consuming invertebrates (which may act as parenteral hosts for *T. gondii*) (Bettioli *et al.*, 2000a, Bettioli *et al.*, 2000b). Additionally, bandicoots, once infected with *T. gondii*, are considered extremely sensitive to development of clinical disease (Bettioli *et al.*, 2000b). Studies on bandicoots in other areas of Australia have indicated a strong association between *T. gondii* infection and reduced longevity (Obendorf *et al.*, 1996). In our study, we found no evidence that seropositive northern brown bandicoots had a different health status to other animals, although one individual was also infected with HV. Without recapture data we are unable to infer survival.

There are reports of seropositivity to *Toxoplasma* in wild brushtail possums, although this species appear relatively resistant to both infection and the development of clinical disease compared to bandicoots (Hill *et al.*, 2008, Ladds, 2009). Eymann *et al.* (2006) found a seropositivity of 6.3% to *Toxoplasma* in brushtail possums from Sydney but other studies found no seropositive brushtail possums in WA and Tasmania (McCutcheon *et al.*, 2007, Hollings *et al.*, 2013). The absence of seropositive brushtail possums in our study may therefore not be anomalous.

The absence of seropositive northern quolls was somewhat unexpected. Although studies frequently show high levels of seropositivity in dasyurids, this taxon is generally considered less susceptible to clinical disease from *Toxoplasma* infection than other marsupial groups (Attwood *et al.*, 1975, Ladds, 2009). Fancourt *et al.* (2014) found a high prevalence of *T. gondii* seropositivity in eastern quolls (*Dasyurus viverrinus*) in Tasmania but no evidence of disease associated with infection, and no effect on longevity of the host. Oakwood and Pritchard (1999) found little serological and no histopathological evidence of *T. gondii* infection in over 20 northern quoll carcasses from Kakadu National Park, retrieved after road kill.

As our study only found evidence of elevated antibody levels to *Toxoplasma* in three northern brown bandicoots and one feral cat (all from Darwin) it is possible that some of these cases were false positives, given the estimated Se and Sp for this testing method (both 0.95). However the fact that all positives came from one location, and were from two species commonly infected with *Toxoplasma* suggests that these results were true positives. Assuming this, our estimated true prevalence of *Toxoplasma* infection in northern brown bandicoots at Darwin is 22.2%, which is note-worthy, particularly in comparison to the other locations sampled. Additionally, a feral cat from Darwin tested seropositive for *Toxoplasma*, providing confirmation that the infection is present in cats in the Darwin region.

Given that seroprevalence for *Toxoplasma* infection was lower in our study than expected we propose two possible explanations, with contrasting implications.

1. It is possible that *Toxoplasma* is acting as a significant pathogen at some, or all, of our study locations, but that the majority of individuals infected with *Toxoplasma* die, or are predated, and are thus removed from these populations before testing.
2. It is possible that the prevalence of *Toxoplasma* infection is low in the target populations, other than in the Darwin region.

In examining scenario one, we consider that northern brown bandicoots would be the target species most likely impacted at a population level by *Toxoplasma* infection, due to known susceptibility of bandicoots to natural and experimental *Toxoplasma* infection (Obendorf *et al.*, 1996, Bettiol *et al.*, 2000a). We consider it less likely that all northern quolls and brushtail possums infected with *Toxoplasma* in Top End populations would have died and been removed from the study, due to their inherently lower susceptibility to disease. If *Toxoplasma* was present across the Top End landscape, and causing mortality in northern brown bandicoots, we would expect to see some level of seropositivity in brushtail possums and northern quolls. Additional information would be required to further investigate scenario one. This could include serostudies of target species in areas where cats have been excluded, and options for retrieving carcasses of target species after death (e.g., as may be achieved through radio-tracking studies) to allow post mortem examination. In the absence of this information, we cannot draw a more firm conclusion on the likelihood of scenario one.

Scenario two assumes that the results of our serological study are a true indication of the prevalence of *Toxoplasma* infection in the study populations and that *Toxoplasma* prevalence is low in all target species other than northern brown bandicoots, and low at all study locations other than Darwin. Whilst *Toxoplasma* cysts may survive for extended periods in the environment, they are sensitive to desiccation (Dubey and Odening, 2001). It may be that the climate of the Top End (with an extended warm dry season) limits survival of *Toxoplasma* cysts in the environment and thus reduces opportunities for marsupial hosts to become infected with this pathogen, compared to temperate areas of Australia, such as Tasmania, where prevalence of *Toxoplasma* infection in free-ranging wildlife is shown to be high (Canfield *et al.*, 1990, Obendorf and Munday, 1990, Bettiol *et al.*, 2000a, Hollings *et al.*, 2013, Fancourt *et al.*, 2014). It may also be that the prevalence of *Toxoplasma* infection in feral cats in the Top End is lower than expected or that the prevalence of feral cats across the Top End landscape is lower than elsewhere in Australia.



Our study indicates higher prevalence of *Toxoplasma* in Darwin than other locations. There are several possible explanations for this. *Toxoplasma* cysts may survive longer in the environment in the Darwin area due to artificially high moisture levels in the soil, as a result of anthropogenic effects such as watering of gardens and playing fields during the dry season. It may be that native mammals close to human habitation and hence presumably close to higher densities of cats (in this case most likely pet cats, but also feral) have higher exposure to *Toxoplasma* cysts. Other studies have suggested that populations of bandicoots closely associated with high densities of cats (such as on urban fringes or areas with significant human populations) are likely to be at higher risk of *Toxoplasma* infection than those where cat populations are lower (Lynch, 2008). In the Top End, it may be that the prevalence of cats in study areas outside Darwin is low compared to the urban area. Further data are required to investigate these hypotheses. It would be valuable, as further information is gathered on cat movement patterns and densities in the Top End, to compare *Toxoplasma* seroprevalence in a wide range of free-ranging mammal species, against both cat prevalence and *Toxoplasma* seroprevalence in cats across the Top End. This would assist greater understanding of the epidemiology of *Toxoplasma* in this region.

The zero seroprevalence found in our study at most locations may reflect a highly virulent pathogen at undetermined prevalence, associated with a high fatality rate and few survivors after infection; low cat prevalence; low levels of *Toxoplasma* infection or shedding in cats in these areas; and/or reduced survival of *Toxoplasma* cysts in these environments. It may be that *Toxoplasma* is having an impact on declining populations in the Top End, either directly or indirectly, however with limited information to hand, we cannot comment further on this. Further studies in areas where cats are excluded, and ongoing serostudies in a wider range of native mammals, will help to develop hypotheses. If feral cat numbers increase in the Top End, we would expect all marsupial species (particularly bandicoots) to be at increased risk of disease from *Toxoplasma*. Although the significance of *Toxoplasma* as a pathogen in Top End mammal populations remains unclear, we continue to consider this pathogen a high priority for further investigation.

## 4.8 Repeat testing

During our study we hoped to conduct repeat testing on individuals, but this was rarely possible because specific sites within locations were surveyed on a less-than-annual basis; permanent marking was not used at many of the study locations; and there were very few mammals trapped at Kakadu National Park, the only location that was revisited several times during the course of our study. As a result, our study had limited opportunity to follow the health and disease status of individuals over time. This information would be a potentially very powerful tool in seeking to further an understanding of the health of Top End small mammal populations.

In general, wildlife disease studies will have increased power if they can be continued over sufficient temporal span with sufficient numbers to allow longitudinal trends to become apparent and to enhance the power of any analyses performed. Due to the inherently dynamic nature of disease, episodic outbreaks may not be detected with short or even medium term studies. When analysing risk factors for disease expression (such as links to climate or ecological variables) long-term studies are often necessary to obtain sufficient data sets and to overcome issues of confounding (O'Brien *et al.*, 2009, Ryser-Degorgis, 2013).

Studies that allow comparison of different groups (e.g., populations undergoing declines versus stable populations or 'case-control studies') may provide valuable information and improve interpretation of results in the absence of baseline health and disease data for wildlife populations (Thrusfield, 2013).



## 4.9 Resilience and susceptibility of Top End populations to disease

Species that have arrived ‘recently’ into the landscape are recognised to have the potential to disrupt ecosystems (Elton, 2000). Additionally they may bring with them novel pathogens, which may cross into and cause disease in endemic species (Daszak *et al.*, 2000, Pedersen *et al.*, 2007, Jakob-Hoff *et al.*, 2014, Tompkins *et al.*, 2015). Black rats, feral cats and pigs, cane toads, humans of European origin and introduced herbivores are all ‘new’ arrivals to the Top End and are all known to act as carriers of disease.

Factors such as increasing global movement of humans and climate change may increase risks of emerging diseases (Daszak *et al.*, 2000, Tompkins *et al.*, 2015). The tropical nature of the ecosystem of the Top End and its geographical proximity to our northern neighbours increases the risk of exotic infectious diseases moving into the Top End (Reid *et al.*, 2001).

Chronic ‘stress’ may result in a reduction of a host’s natural ability to fight disease. Stress-related effects may be linked to factors such as reduced food and shelter, increased climatic stress and pressure from conspecific animals, competitors and predators. These effects may be manifest physiologically as reduced functionality of the individual’s immune system (Padgett and Glaser, 2003, Ladds, 2009). ‘Stress’ or concurrent disease may also operate at a molecular level and alter the individual’s anti-oxidant status and hence its ability to cope with additional stressors in its environment (Cooke *et al.*, 2003, Rabus *et al.*, 2008). Reduced genetic variability, which is often seen as populations diminish and become more fragmented, may also result in reduced immune competence (Hedrick, 2004, Spielman *et al.*, 2004). Together, these factors may be considered to impact the ‘resilience’ of both individuals and populations, otherwise described as their ability to stay healthy, reproduce and thrive in the face of increasing pressures and environments undergoing change.

The potential for disease to act on threatened and small populations is well documented (Lafferty and Kuris, 2005, Schloegel *et al.*, 2006, Pedersen *et al.*, 2007, Kriger and Hero, 2009) and disease is of heightened concern for threatened species, where populations are small and isolated and where genetic variability is diminished (Pedersen *et al.*, 2007, Suzán *et al.*, 2012, Heard *et al.*, 2013).

Within the Top End, the cumulative and synergistic effects of apparently mild stressors and threats such as sub-clinical or non-lethal disease, low-level climate change, minor vegetation changes and low-level predatory threats may result in significant ecological impacts. The significant, and likely cumulative, effects of changed fire regimes and introduced flora and fauna are recognised (Ziembicki *et al.*, 2015). Small mammal populations in the Top End, and in particular those where declines are occurring, would appear to be highly susceptible to the threats posed by disease, and it is likely that these threats will continue to increase over time.

## 4.10 Project limitations

Aspects of this study may limit the ability to draw meaningful conclusions on the role of disease in mammal declines. These include:

- investigating causes after a decline has occurred, as infectious agents involved in the initial wave of declines may no longer be present
- lack of existing baseline information on health and disease in native mammals of northern Australia against which to interpret our findings
- the limited duration of this study and, in some species or locations, limited sample sizes available
- lack of opportunity to study target species in areas where feral cats were excluded
- limited quantity or quality of samples (e.g., blood and faeces) available from small species, especially native rodents, due biological difficulties in safely and humanely collecting blood
- a project design focused, for logistical reasons, on sampling predominantly during the dry season, which may reduce ability to detect seasonal or other cyclical changes in disease prevalence
- other than northern brown bandicoots, all other target species were only sampled in reasonable numbers at two of five study locations, making comparisons between species and locations challenging
- limitations in availability and applicability of current diagnostic tests for pathogens of concern in wildlife species including test sensitivity and specificity in target species
- potential sampling biases, including selection bias created by using trapped animals for investigation. Additionally, to balance sex-ratios we selected individuals for examination and sampling based on their gender

## 4.11 Additional project outcomes

In addition to undertaking an extensive investigation of health and disease in small mammal species undergoing decline in the Top End, this study:

- established protocols and processes for undertaking wildlife disease investigation in small mammals in a tropical environment with limited access to facilities and services
- increased the awareness of local staff around recognition and reporting of disease processes
- expanded multi-disciplinary collaborations (of note between ecologists and veterinarians), in particular in assessing the impacts of disease on biodiversity, and highlighted the importance and the challenges of including disease as a factor when assessing impacts on wildlife populations
- developed strong and valuable collaborations with a broad range of researchers and diagnosticians in Australia and NZ
- raised awareness of the population declines of mammals in the NT throughout the scientific and wider community, both within Australia and globally
- assisted in supply of diagnostic and research material and data to scientists working in diverse areas of wildlife health and disease
- archived a large amount of biological material for use in future research.

## 5 Conclusion

### 5.1 Is disease a factor in declines of small mammals in the Top End?

Our study did not find compelling evidence that a single pathogen is responsible for the decline of small mammals in the Top End of the NT. However, and importantly, we identified the presence of several potentially significant pathogens in study populations. These pathogens are known to be associated with disease in wildlife populations, particularly in the presence of environmental and physiological stressors. Additionally, we found interesting location associations for certain pathogens, syndromes and variables that may reflect important differences in population health between locations. These findings warrant further investigation. The recognised limitations of the study may hinder investigation of potentially complex interactions between pathogen presence and population declines.

We conclude that in general, the health of the animals examined appeared largely normal and we found no evidence for significant ill health in the populations we examined. However, a lack of evidence for a plausible pathogen does not remove infectious diseases from the list of potentially significant factors driving population declines in the Top End. There is great complexity in ecological systems and disease may be playing a synergistic role, along with other factors (e.g., toxoplasmosis may increase susceptibility to predation, linked to reduced protective habitat, as a result of changed fire regimes) (Legge *et al.*, 2011).

We have greatly expanded the knowledge of health and disease in small mammal populations in the Top End of the NT. Despite this, the information obtained in this study should only be used to make informed hypotheses about the contributory role of infectious disease in the Top End landscape. As demonstrated earlier, a robust understanding of disease impacts within wildlife populations may take decades to develop, even when there is clear evidence that a disease is present e.g., facial tumour disease in Tasmanian devils (McCallum, 2008). When effects of disease are subtle or obscured by other threats, the true impacts of disease may be much harder to discern (Tompkins *et al.*, 2002, Skerratt *et al.*, 2007, Kriger and Hero, 2009, Miller *et al.*, 2012).

Although our studies did not reveal clear support for the hypothesis that infectious disease is impacting small mammals at a population level in the Top End, we conclude the following:

- There is evidence that several infectious diseases, capable of impacting population health, are circulating in Top End small mammal populations.
- Regardless of whether infectious disease is currently impacting these populations, small mammal populations in the Top End should be considered highly vulnerable to the potential impacts of disease and there is a strong chance that disease could emerge as a significant factor in the future.
- Ongoing disease investigation should be undertaken in Top End small mammal populations of concern, to increase knowledge above current levels; to improve temporal data; to maintain and build current capacity in this area; and to maximise opportunities for detection and response to new disease threats as they emerge.

## 5.2 What do we recommend?

We recommend that disease should continue to be a concern and of interest to those involved in research and management of small mammal population declines in the Top End. As protocols are now well established and thoroughly documented, and capacity to undertake this work has been developed, we believe that circumstances are well placed for disease monitoring to continue into the future. We recommend that resources, as available, should be devoted to continue targeted, strategic research on health and disease in small mammal species and populations of concern in the Top End. Increasing knowledge and awareness of disease within agencies involved in research and management of declining wildlife populations will increase the likelihood that disease outbreaks will be detected and responded to appropriately. Ongoing collaborative efforts between NT DLRM and research institutions, such as the Conservation Medicine Program within the School of Veterinary and Life Sciences at Murdoch University, may facilitate research and enable continued opportunities for work funded through competitive grants, industry sponsorship and student research projects.

Our overarching recommendations are that ongoing disease research (in the target species and at the established study sites) should continue in the medium to long term (five to 20 years). At a minimum, two to three study sites could be selected, with an aim to undertake ongoing disease surveillance every two to three years. Such efforts, in combination with the collection of ecological data, would provide additional spatiotemporal value to the existing dataset and allow for more robust interpretation of results. This ongoing work will be vital to identify any significant changes or emergences in disease status that might occur over time. Future research in this area will be governed by funding, logistical support and long-term research priorities.

The following prioritised recommendations are drawn from our analysis of data:

### 5.2.1 High priority recommendations

- Increased use and development of techniques such as mark-recapture, thus allowing studies to follow individual animals over time. This would allow for longitudinal, linked studies of an individual's health, disease, pathogen, serological and survival status, which are all important when seeking to understand the epidemiological significance of disease.
- Inclusion of studies allowing individuals to be tracked free of risk from introduced predators, and studies which increase chances of detecting sick and recently deceased individuals. Examples include studies within predator exclusion zones and use of remote tracking (e.g., radio- or satellite-tracking). The ability to monitor an individual's disease status against health and survival over time, including if and when seroconversion occurs and how seropositivity affects survival and health, will greatly improve interpretation of current and future data, as will improved ability to retrieve sick animals or fresh carcasses for investigation. There will be an increased chance of detecting and correctly interpreting the significance of many potentially significant pathogens, if the risk of predation is reduced.
- Continued opportunistic and targeted collection and storage of sera and other samples from target species and other species of concern, for future collaborative studies.
- Engagement of community to increase awareness of small mammal declines, the possible role of disease and the need for opportunistic collection of suitable samples.
- Exploration of hypotheses developed from *de novo* molecular testing and investigation of potential pathogens of interest emerging from this work.

- Long-term (5+ years) seroprevalence studies for *Toxoplasma gondii* in selected target populations and species, allied to studies on the presence and prevalence of cats, to further elucidate the potential role of this pathogen in population declines. Research should include studies in areas where cats are excluded, to allow observation of the impact of disease in the absence of predatory pressures and scavenging of carcasses.
- Collection of data on cat presence and prevalence across the Top End and extension of studies to look for serological and histopathological evidence of *T. gondii* infection in cats, and serological evidence of exposure to *T. gondii* in a wider range of native mammals (e.g., macropods) across the broader Top End landscape, to better understand the epidemiology of this pathogen in the Top End environment.
- Ongoing collection and analysis of basic morphometric data on target species at different locations, to determine if the observed trend of larger animals at Kakadu National Park compared to other study locations continues over time and if so, to look further at the possible reasons for this.
- Inclusion of studies to assess 'fitness' of individuals and the role of chronic or sub-acute stress through assessment of excreted cortisol analysis and emerging technologies, such as measurement of anti-oxidant capacity. Such variables may provide an indication of population-level compromise in immune-competence and general health status. Such compromises may arise from numerous causes (e.g., nutritional, predatory or conspecific pressures) and may be one factor in a multifactorial population-level effect.
- Ongoing risk assessment and mitigation procedures to minimise disease transmission risks during animal trapping and handling and prior to any animal movement. Additionally, appropriate zoonotic disease risk minimisation processes should be followed by personnel working with wildlife.

### 5.2.2 Medium priority recommendations

- Ongoing comparison of health and disease status of different groups (e.g., populations undergoing declines versus stable populations; predator excluded zones versus Darwin locations) to improve interpretation of significance of findings.
- Continued monitoring for clinical signs of herpesvirus (such as cloacal or other mucosal lesions) in target populations or deaths possibly associated with HV. Additionally, further testing for HV, via PCR, on populations of concern in approximately 5 years, to monitor for any changes in prevalence or shedding rates, which may be indicative of changes in population fitness. Sampling could be restricted to the oropharynx. Serological tests, if available, should also be considered.
- Continuing and expanded (to other species known to be involved in the epidemiology) serosurveys to gain wider understanding of *Coxiella burnetii* epidemiology in the Top End, with the use, where possible, of ELISA for serological studies. Investigation of *C. burnetii* by PCR studies, of both mammals and ticks, as this may provide an alternative method of gauging exposure and carriage of this organism.
- Ongoing studies to investigate potential links between both protozoal haemoparasites and enteric protozoa and disease, including analysis against haematological variables. Surveillance for trypanosomes, *Babesia* spp., *Hepatozoon* spp., *Giardia* spp. and *Cryptosporidium* spp. in Top End mammal populations, utilising where possible established collaborative research arrangements, should be continued.

- Continued monitoring for the presence and prevalence of skin disease in Top End small mammal populations and appropriate investigation (photography, skin scraping and biopsy for histology and culture) if any changes or new presentations are observed. This should include monitoring and investigation of lesions suggestive of pox- or papilloma-like skin disease and observation of the presence and severity [using the four point scoring scale described by Hufschmid *et al.* (2010)] of rump wear in all monitored brushtail possum populations in the Top End. Any changes in prevalence or severity in skin disease, and in particular rump wear, over time may be considered as indicators of potential population level changes in stressors and could be considered a trigger to look more closely at the health of populations.
- Obtaining additional information on GIT helminths in declining populations via alternative investigative methods, as some species of GIT helminths may cause significant disease, in particular when acting synergistically with other factors. Opportunistic collection and processing of gastrointestinal tracts from carcasses, in species of interest, processed according to the specific protocol developed for this project (contact authors for details), followed by identification of helminths by an experienced parasitology taxonomist is recommended. Additionally, histopathological investigation of suitable carcasses could be undertaken to look for evidence of disease associated with GIT parasites.

### 5.2.3 Lower priority recommendations

- Ongoing low-level surveillance for *Salmonella* spp. in Top End populations of concern, to monitor for presence, prevalence, serovars and any associations with environmental or host stresses. Surveys could be undertaken every five years.
- Continued serosurveillance for *Leptospira* spp. in target populations, which will improve understanding of which, if any, *Leptospira* serovars are circulating in Top End small mammal populations. Close attention should be paid to any emerging evidence of novel *Leptospira* infections in Top End populations.
- Continued observational studies for the presence of EMCV in Top End populations of concern, including appropriate investigation of carcasses and archiving of sera, as available, for future serosurveys. At a broader level, investigation of seroprevalence in wild pigs in the Top End, to determine if this species is acting as a reservoir for the pathogen, could be undertaken.
- Ongoing investigations recording the presence and prevalence of microfilaria in peripheral blood samples (as testing is easily achieved and has minimal cost implications) and identification of the species of filaroid nematodes involved in each host species, by an experienced parasitologist.
- Continued collaborative investigations into Barmah Forest and Ross River virus epidemiology in the Top End environment.

Our study has made significant inroads into understanding health and disease in four key small mammal species in the Top End. It is our recommendation and hope that this work can be continued, albeit perhaps at a less intensive level, over the next decade or two, to allow a fuller opportunity to assess the impacts of disease on these declining populations. We hope the data we have gathered and the analysis we have undertaken on health and disease in these species and populations will help to inform and focus future research and management activities.



# Appendix 1 - Infection, disease, diagnostic testing and interpretation

## Infection and disease

The presence of a pathogen within an individual does not equate to disease. Infectious agents are often a normal part of the biology of wild animals and may be present without causing significant disease.

A range of outcomes are possible when an animal becomes infected with a pathogen. Individuals may:

- carry or be infected with a pathogen, without developing disease (referred to as sub-clinical or asymptomatic infection)
- develop mild or moderate illness from which they recover
- develop a persistent infection
- develop a severe illness resulting in death

Disease, when present, may have direct results in increased mortality or reduced fecundity or may exert more subtle effects which predispose individuals to death through, for example, increased predation rates (Wobeser, 2006). Mortality may be directly due to the infectious disease, or because the individual was compromised by the illness to such an extent that death occurred by other means (such as starvation or predation). In some cases of persistent infection, the animal recovers to good health, but remains as a 'carrier' of the infection, whereby it carries and sheds the pathogen but is not in itself unwell.

Many factors influence whether an individual becomes sick, how sick it becomes and whether it lives or dies following pathogen exposure. The most important dynamics are between the host (the exposed animal), the environment in which it lives, and the pathogen itself (Figure 28).

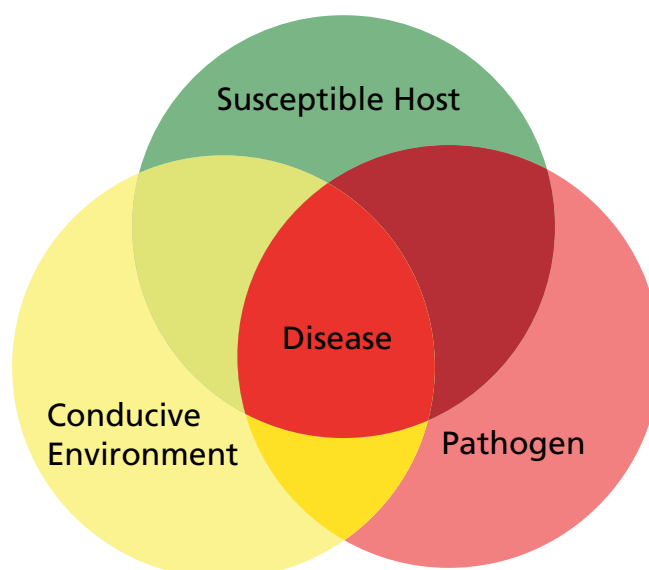


Figure 28: The host/ pathogen/ environment relationship.

These three factors may interact in the following ways:

**Host** – age, health, reproductive status, genetics, acquired immunity, concurrent diseases and inter-current stressors (e.g., social competition for nest sites) may influence the likelihood of an individual succumbing to a pathogen and the severity of the resulting disease. Juvenile and aged animals are generally considered to have less competent immune systems. Stresses may have a direct effect on the immune function of the individual.

**Environment** – factors such as climate and weather, food and other resource availability, habitat disturbance, predatory pressure and anthropogenic effects may influence the way a disease affects individuals and populations.

**Pathogen** – factors such as how the pathogen is transmitted, how long it can survive in the environment and how quickly it kills its host all influence the way pathogens affect and move within a population.

Pathogens vary in their ‘species specificity’ (how narrow or wide the range of host species in which they cause disease). Some pathogens cause disease in a wide range of animal species. Other pathogens are known to be highly host-specific and only infect a narrow range of species. *Toxoplasma gondii*, the protozoal parasite responsible for toxoplasmosis, is an example of a pathogen with a very wide species host range, as all mammals are considered susceptible (Hill and Dubey, 2002). Wallal virus (causing blindness in wild kangaroos) is an example of a narrow host pathogen, where laboratory testing has confirmed that only macropods are susceptible to disease (WHA, 2010).

Some host species are recognised as carriers of disease or maintenance hosts. These species become infected with a pathogen with little or no resulting disease and provide an environmental ‘reservoir’ for a pathogen. They may also act as ‘amplifying hosts’ allowing the pathogen to reproduce and be shed in vast numbers. Reservoir or carrier species are critical elements in disease-induced declines or extinctions as they may allow a disease to persist beyond population thresholds described in disease theory (Lloyd-Smith *et al.*, 2005). Basic disease theory states that as populations decline, infectious diseases are expected to ‘die out’ due to a reduction in the number of susceptible individuals and the number of host interactions, to such a point where the disease can no longer be transmitted sufficiently to be sustained. Reservoir species may continuously reintroduce disease into threatened or declining species even when small population thresholds are reached, and these are the conditions under which disease-induced extinction can occur (de Castro and Bolker, 2005).

Rodents, in particular the black rat, are well recognised as carriers for many infectious diseases including leptospirosis and encephalomyocarditis virus which may spread to other, susceptible host species. Feral or domestic cats and dogs may also carry and shed pathogens, in particular *T. gondii* in cats. Because these hosts are relatively recent arrivals to Australia, it is plausible that they may carry and spread diseases to which the native fauna are not historically exposed. In general, individuals or populations that have not been previously exposed to a pathogen are considered more vulnerable to the effects of infection than non-naïve populations. Many pathogens arrived in Australia with European settlement and the associated arrival of domestic and feral animals. Native Australian fauna did not evolve in the presence of these pathogens, and it is reasonable to assume that the host susceptibility of native species is therefore greater.

Pathogens vary in their survivability outside the host, which also influences disease dynamics e.g., pathogens which have infectious stages that can survive for months or longer in the environment (such as *T. gondii*); pathogens which require close contact between individuals for disease transmission; and pathogens which have evolved to survive and be transmitted by biting insects (e.g., mosquitoes, biting flies and ticks), also called 'vector borne' diseases. Such pathogens may be a significant factor of infectious disease in a tropical environment and due to the seasonality of vectors, may show cyclical activity during the year. Other pathogens, independent of vector transmission, may also show seasonality, influenced by environmental temperature and moisture.

It is well recognised that immunocompromise (a less effective or depressed immune system) may play a role in the development of clinical disease states in all mammal species (Padgett and Glaser, 2003, Ladds, 2009). Factors associated with free-ranging existence (e.g., resource limitation, climatic factors, inter-species or intra-species competition, aggression, reproduction, age, predator pressure) may contribute to stress and hence immunocompromise such that clinical disease may develop (Acevedo-Whitehouse and Duffus, 2009). Immunocompromise may also be related to inter-current diseases or co-infection with multiple pathogens. More than one pathogen influencing the population may place it at increased risk of disease-related decline.

## Testing for disease

There are a wide variety of methods used to test for disease. In general, a specific pathogen will have a particular group of tests that are recommended for use in diagnosis or investigation of this agent. A variety of biological samples may be used to test for pathogens and disease. Generally each test requires a specific type of sample (e.g., mucosal swab, serum, faeces).

### Sensitivity and specificity of tests

There may be significant variation in the accuracy of diagnostic tests. No diagnostic test for a pathogen can be considered 100% accurate. Diagnostic test accuracy is described for two different parameters:

**Test sensitivity (Se)** is the likelihood that a truly positive individual will return a positive test result.

**Test specificity (Sp)** is the likelihood that a truly negative individual will return a negative test result.

Many tests have relatively high sensitivity but low specificity (i.e. they can accurately detect a positive individual, but many negative animals also test positive; referred to as false-positives). The occurrence of false positive results is a concern for any disease surveillance or research, including that involving wildlife. Whenever possible, best practice requires the use of a secondary test for positive results, to confirm their status as true positives. In wildlife disease investigations, however, there are generally limited options for secondary tests.

Testing for infectious agents in animals can be classified into two broad categories:

1. Tests which look for direct evidence of the pathogen in a biological sample
2. Tests which look for evidence that an animal has been previously exposed to a pathogen

## Tests for direct evidence of pathogens

Tests that look for direct evidence of pathogens in the biological sample involve processes to identify the agent of disease itself and reflect the infection status of an individual at a given point in time. Tests may include:

- sampling and culturing for growth of pathogens (generally used for bacteria; viral culture is a specialised and difficult field)
- techniques such as PCR (polymerase chain reaction) to look for molecular evidence of DNA or RNA from pathogens (used for many different types of pathogens, including bacteria, viruses, parasites and protozoa)
- direct observation of pathogens (gross or microscopic examination)

All these tests require the pathogen to be present in the sample for a positive result. Inaccuracies may result from poor or incorrect sampling technique, inappropriate samples being collected, poor sample storage, lab errors, low concentration of pathogen in samples or lack of observational skills and experience in lab staff.

Test results must also be appropriately interpreted for the pathogen, test type, host species and circumstance. A direct test only provides information on whether the individual is currently infected (and whether the sample contained sufficient pathogen to yield a positive result). It will not provide information on whether the individual has previously been infected but has now cleared the infection, and it does not provide information on whether the animal is suffering disease as a result of the infection.

## Tests for indirect evidence of pathogens

Indirect tests identify evidence that the animal has experienced prior exposure to the infectious agent and reflect the history of exposure of the host to the pathogen. These tests generally look for presence of antibodies in blood, which rise in response to an individual's exposure to a specific infectious agent. These serological tests require serum (the liquid portion of blood, with all red and white blood cells removed). Serum must be kept frozen prior to antibody tests being performed and a minimum volume of serum is required for each individual test, generally 0.1 ml. Although modern technologies have allowed for multiplexing (where multiple serological tests can be run concurrently from the one small volume of serum), such technologies are not commonly available for wildlife testing in Australia.

Inaccuracies in serological tests can result from poor quality serum, poor storage of serum, poor specificity of the test for the host species and cross reactivity with similar but different pathogens. Serological tests will only detect antibodies for as long as they continue to circulate in the host's system. In many cases, antibody response to infection by a pathogen wanes over time. In most cases in wildlife species, the length of duration of persistence of antibodies following infection is not known.

Some serological tests can be run on serum from any host mammal while other types of serological tests are specifically designed for the host species. In many cases, commercially available serological tests for pathogens in Australian labs have been developed for domestic animals. Few serological tests have been developed specifically for wildlife species, and most of the serological tests used for Australian wildlife fall into the category of those types of tests which are not host-species specific.

In any disease analysis there are chances of false positive results (i.e. detection of disease when it isn't actually there) and false negative results (i.e. failure to detect disease when it is present). In this study, we factored those variables into our analyses and determined at what prevalence (percentage of diseased individuals in a population) each disease may have been present, without being detected within the sample size.

## Interpretation of test results

Interpretation of test results and assessment of disease status of individuals and populations is a complex process that relies on a multitude of inputs and sound clinical judgment. Interpretation of test results must be undertaken with care in wildlife studies, where baseline knowledge is often limited, and tests have not been validated for the species under investigation. Sensitivity and specificity of tests have often not been calculated for wildlife species and must be inferred or assumed based on expert knowledge.

Sample size has a significant influence on interpretation of tests results. Studies to look at presence and prevalence of a disease in a population will be influenced, among other factors, by the number of individuals sampled. Small sample sizes will lower the level of detection in the population and may make interpretation of results more challenging. When it comes to interpreting risk factors against the outcome of disease (e.g., does sex or age influence the likelihood of disease in this species), a failure to detect an effect despite one existing (Type II Error) is common with small sample sizes and is a limitation of wildlife studies in general (O'Brien *et al.*, 2009).

A lack of evidence for a pathogen (i.e. a negative test) within a population does not prove that pathogens are absent from that population. If the sample size is small, a negative result may mean the disease is present, but the prevalence is below that able to be detected with that sample size. Tests for direct evidence of a pathogen will only be positive if the individual is shedding the pathogen at the time of testing, and in the sample collected. Many pathogens are shed intermittently (e.g., *Salmonella*) and some are shed only during times of stress (e.g., herpesviruses). In other pathogens, infection with the pathogen is transient (e.g., influenza virus) and the individual will only test positive during the period of active infection. Tests for serological evidence of exposure to a pathogen will only be positive for as long as the individual continues to have an immune response following infection. In most cases, immune responses wane weeks to months after infection, and in many cases the duration of persistence of immunity is not known for wildlife species and must be extrapolated from knowledge of domestic species. However, animals infected with a particular pathogen may die before an immune response can be manifested, and if all infected individuals die, no animals in the population will show a serological response to the pathogen.

Given the complexities associated with disease investigations in wildlife populations outlined above, a lack of evidence for a plausible pathogen does not remove infectious diseases from the list of potentially significant factors affecting a wildlife population.

Interpretation of each set of disease data requires an understanding of the influence of the test characteristics (Se and Sp, and what the test tells us about the host status), and the ecology of the disease (e.g., transmission characteristics, likely impacts in the host species, likely presentation or detection in a population).

Low levels of prevalence must be interpreted with caution. Most tests are not 100% specific (i.e. a percentage of truly negative individuals will test as positive because the test is not 100% accurate) and low prevalence levels must be interpreted against known or assumed Se and Sp.

## Appendix 2 - NERP - Small Mammal Disease Investigation protocols

The list shows the document number and date of the most current version of each protocol developed for the project.

Document number	Document name	Version Number	Date
1	Overview of field and laboratory procedures	V3a	Jan-14
2	Sample identification protocol	V2	Aug-14
3	Clinical exam in the field	V3	Oct-13
4	Summary sample collection of animals in the field	V6	Jan-14
5	Blood collection	V2	Feb-15
6	Herpesvirus sampling	V3	Oct-13
7	Protozoal investigation sampling	V4	Oct-13
8	Faecal endoparasite investigation sampling	V3	Feb-15
9	Stress studies sampling	V2	Oct-13
10	Miscellaneous sampling protocol	V1	Oct-13
11	Non-invasive field sampling faeces and ectoparasites	V1	Aug-13
12	Next generation pathogen discovery sampling	V2	May-14
13	Brain and muscle collection for <i>T. gondii</i> collaboration	V1	Jan-14
14	Emergency GA response	V1	Jul-13
15	Sample processing in the field	V2a	Oct-13
16	Serum processing and storage	V2	May-14
17	Cleaning and disinfection in the field	V2	Oct-13
18	Field anaesthesia machine care instructions	V1	Jul-13
19	Post mortem examination and sampling	V2a	Oct-13
20	Update on sampling	V1	May-14
21	Sampling protocols for private veterinary practitioners	V1	Jan-14
22	Summary sample collection for private vets	V1	Jan-14



## Appendix 3 - NERP - Small Mammal Disease Investigation field data sheet

Date:		Scribe:		Sample no:	
Handlers:		Vet:	Survey location:	Rain /precipitation?	Y N
GPS coordinates:		Trap point:		Time trap checked:	
Microchip		Normal at release?	Y N	Release time:	
Physical data		Morphometrics		Weights	
Species		Head length	mm	Animal+bag	g/kg
Sex	Male Female	Foot length	mm	Bag alone	g/kg
Age class	Juv SubAd Adult	Head width	mm	Animal	g/kg
State of Alertness	Trap: 1 = unresponsive 2= reduced response 3= responsive GA:	Demeanour score	Trap: 1 = Quiet 2= Agitated 3 = Highly agitated GA:		
Health Status	Normal Abnormal	Pouch: Non parous Parous PY Suckling but no PY Elongated teat YAF		PY Unfurred, lightly furred, Pouch emergent	
Body Condition	1 2 3 4 5	No. of PY		PY gender	M F Unk
Repro. status	Inactive	Pouch taped?	Y N	PY crown-rump	mm
Scrotal width	mm				
Sample collection		Time collected:			
Max blood vol. for collection	ml	Ectos collected	Y N		see over
Blood collection site		Faeces	Y N	Formed /unformed	Wet / dry
EDTA #1 ml #2 ml		Swabs (viral)	Urogenital <input type="checkbox"/> Nasal <input type="checkbox"/>		
Plain blood ml			Conjunctiva <input type="checkbox"/> Oropharynx <input type="checkbox"/>		
Blood smears (4)	X	Fur pluck <input type="checkbox"/> Fur clip <input type="checkbox"/>		From hip, wrap in foil	
Microhaematocrit Y N Fresh EDTA		Urine: ml How collected:			
Whatman paper Y N X		Skin scraping (details)			
Total blood volume collected	ml	Viral discovery swa	Rectal	Other	
Ear biopsy (DNA) Y N		Other skin biopsy (details)			
Rectal swab culture Y N					
Other culture swabs Y N		Other samples			
Photos taken Y N (details)					
Anaesthetic data		ETT size:			
Start Ox:	Start Iso:	Iso. off:	Ox off:	Recovery:	
Time:				Medications	
Iso %				Drug; dose, route; time given	
Ox rate (L/min)					
SpO2 %					
Heart Rate					
Resp rate					
Temp. C				SQ fluids	ml <input type="checkbox"/>
Notes:					

<b>Physical exam</b>	Under GA	Conscious		
<b>General comments</b>				
<b>Skin/ coat</b>	Normal	Abnormal:	1    2    3	Acute    Chronic
<b>Comments</b>				
<b>Ectoparasites</b>	Total ecto burden	0 (none)	1 (low)	2 (medium)    3 (high)
<b>Comments</b>			<b>Location</b>	Burden: 0   1   2   3   collected
	Ticks			
	Fleas			
	Lice and nits			
	Other			
<b>Head incl eyes, ears mouth</b>	Normal	Abnormal	<b>Tooth age wear:</b>	
<b>Comments</b>			None    Mild Moderate    Marked	
<b>Abdomen and Chest</b>	Normal	Abnormal		
<b>Comments</b>				
<b>Cloaca genitalia pouch</b>	Normal	Abnormal		
<b>Comments</b>				
<b>Tail, limbs, toes &amp; claws</b>	Normal	Abnormal		
<b>Comments</b>				
<b>Sample processing</b>	(or record these times on log sheet)		PCV	TPP
	<b>Time</b>		<b>Time</b>	<b>Serum aliquots</b> running total
Freeze viral discovery swab		Spin whole blood		x 0.1 ml
Freeze faeces endo		Harvest serum		x 0.2 ml
Freeze faeces #2 (stress)		Freeze serum		x 0.3 ml
Fix blood smears		Freeze fur		x 0.4 ml
Harvest AntiOx Plasma		Fix faeces ethanol	Serum quality	
Freeze EDTA blood		Freeze blood clots		<b>Total serum</b> ml
<b>Notes</b>				
			<b>Sample no:</b>	

## Appendix 4 - Further information on pathogens not prioritised for investigation

Information in this section has been summarised from Vogelnest and Woods (2008) and Ladds (2009), unless indicated otherwise.

### Notes on other bacteria not prioritised for investigation

***Actinomyces* spp.** are probably involved as one of the factors in exudative dermatitis (severe 'rump wear') in brushtail possums.

***Bartonella* spp.** are intracellular bacteria which may be found in blood cells. Infection has been found in macropods, although the significance of this is not known. Bartonellosis is an emerging zoonotic disease and more information in this area is desirable.

***Bordetella bronchiseptica*** is a bacterium associated with respiratory disease in captive and free-ranging koalas and captive wallabies. It has not been reported as a pathogen in target taxa.

***Burkholderia pseudomallei*** is the bacterium responsible for melioidosis, a significant infection of humans (and animals) in the monsoonal north of Australia. The bacteria are found in the environment and infection, which has a seasonal occurrence with high rainfall, is via ingestion, inhalation or contamination of skin wounds. Disease may occur in outbreaks associated with stress and signs include generalised malaise, septicaemia, abscesses and neurological signs. Serological surveys have shown that infection is geographically widespread but the disease is not considered common. There are few reports of disease associated with melioidosis in native mammals. Antibodies have been detected in a range of Australian mammal species including macropods, native rodents and possums. Rodents may be carriers of the disease.

***Chlamydia* spp.** are recognised as a significant pathogens of koalas, however there is emerging information about disease associated with chlamydia infections in other Australian mammals, including western barred bandicoots (Warren *et al.*, 2005). *Chlamydiales* in mammalian wildlife are not considered zoonotic.

**Clostridial disease: Tetanus** is seen occasionally in macropods but has been rarely reported in other species. **Tyzzler's disease** (caused by *Clostridium piliforme*) has been reported in a wide range of Australian mammal species but almost always in captive juveniles. It generally results in sudden death.

***Chromobacterium violaceum*** is a bacterium found in soil and water in the tropics and may, rarely, cause serious disease in humans. It has been reported on one occasional in a captive, juvenile macropod.

***Erysipelothrix* spp.** have been rarely reported as causing disease in northern brown bandicoots and other Australian mammal species.

***Escherichia coli* and related species:** These bacteria are ubiquitous in the environment and are commonly found in the GIT of healthy animals. Some strains may be much more pathogenic than others. They are reported to cause primary disease in hand-raised macropods but disease is not widely reported in other Australian mammal species.

***Helicobacter* spp.** are GIT-associated bacteria that have been identified in brushtail possums, quolls and bandicoots. Little information is currently available but it appears they may be associated with GIT disease in some individuals.

***Listeria* spp.** infection is reported in a wide range of Australian mammal species, notably the *Antechinus* where it is associated with post mating die-off in males and is related to stress.

**Lumpy jaw** in macropods is a multifactorial disease, however the presence of key bacterial species are required. Lumpy jaw is not commonly reported in species other than macropods and is rarely seen in free-ranging mammals.

***Mycobacteria* spp.** A variety of *Mycobacterial* infections have been described in a wide range of Australian native mammals. In the most part, these organisms are present in the environment and infections are most commonly reported in captive animals, where exposure to organisms can be higher than in a wild situation, although very occasional reports describe disease in free-ranging animals. Infection has been reported in macropods, possums (including brushtail possums), bandicoots (including northern brown bandicoots) and dasyurids (including quolls). Infection is considered to be clearly associated with stress and in many cases, captivity. In contrast to NZ, infection of brushtail possums with *M. bovis* has not been reported in Australia and *M. bovis* was eradicated from Australia in the 1970s. *Mycobacterial* infection may develop through contamination of open wounds; skin lesions and abscesses in organs are the most common manifestations. *Mycobacterial* disease is chronic in nature and difficult to diagnose. Infections almost always result in the death of the individual if untreated. Treatment is difficult and requires prolonged medication.

Disease associated with ***Pasteurella* spp.** has been reported in a range of Australian mammal species including bandicoots, possums and rodents and is generally associated with circumstances of stress. Infection is a common sequel of cat bite wounds and may result in abscesses, pneumonia, septicaemia and death.

***Proteus* spp.** have been frequently reported in a range of Australian mammal species but are considered opportunistic pathogens.

***Pseudomonas* spp.** are opportunistic bacteria; infections may occasionally cause serious disease in wildlife including in macropods (especially juveniles) and koalas. The bacteria may cause septicaemia and has been reported associated with dermatitis in a captive brushtail possum.

***Staphylococcus* spp., *Streptococci* spp., and *Corynebacteria* spp.** are bacteria reported in a wide range of Australian mammal species, but infections are generally opportunistic and associated with captivity. *Staphylococcus aureus* is associated with swollen paw syndrome in ringtail possums and may result from infection of traumatic wounds in free-ranging animals. Reports of disease generally involve single individuals.

***Streptobacillus moniliformis*** is the cause of rat bite fever in humans (a zoonosis) and has been isolated from healthy rodents in northern Qld. It has been associated with sudden death in captive spinifex hopping mice (*Notomys alexis*).

***Yersinia* spp.** are often found in the GIT of healthy animals, including macropods and possums. Disease may occur sporadically in individuals or in an outbreak situation. Infection may cause serious disease in situations of stress, especially in captive animals and disease may occur concurrently with toxoplasmosis. Clinical signs include severe GIT disease, septicaemia and death, or chronic abscesses.

## Notes on fungi not prioritised for investigation

**Adiaspiromycosis** is a fungal infection causing chronic respiratory disease in wombats; it is also reported in brushtail possums but only in NZ. Infection and disease have not been reported in target taxa in Australia.

**Aspergillus spp.** are spore-forming fungi that are ubiquitous in the environment. Disease occurs most commonly in immune-compromised individuals when they inhale the spores. Respiratory disease has been seen in captive macropods, but has not been reported in target taxa.

**Candida spp.** are yeasts which cause localised and GIT disease, often in hand-raised individuals. The disease is not commonly reported in wild mammals and rarely in the target taxa, even in captivity.

**Cryptococcus spp.** are fungi; the spores are found in the environment, especially associated with species of Eucalyptus trees. Disease is most commonly reported in koalas (both captive and free-ranging) but has also been reported, more rarely, in macropods, possums (including brushtail possums) and native rodents. In koalas, infection is considered opportunistic and is related to immunocompromise. Disease may manifest as neurological and systemic illness.

**Ringworm:** the fungi responsible for ringworm (a common skin disease and a zoonosis) have been reported in a range of species including macropods and brushtail possums. Disease is generally associated with a captive setting and a level of immunocompromise.

## Notes on other protozoa not prioritised for investigation

**Intestinal, hepatic and renal coccidiosis** is common in herbivorous marsupials but not dasyurids. *Coccidia* spp. are considered highly host specific (each species is found only in one host genus or species). There are often several different coccidia species found in each host species, mostly from the genus *Eimeria*. In most Australian native mammals, including our target species there is no, or very little, recognised disease associated with the presence of coccidia. Infection has been reported in brushtail possums and eastern barred bandicoots, but there are no associated reports of clinical disease.

**Klossiella spp.** are coccidian protozoa which parasitise the kidneys of a wide range of mammals worldwide. They have been reported in Australia in bandicoots, rodents and possums but not in the brushtail possum. The presence of *Klossiella* spp. is considered an incidental finding.

**Leishmania spp.** Infection with this protozoa has been described in captive red kangaroos (*Macropus rufus*), northern wallaroos (*M. robustus woodwardi*), black wallaroos (*M. bernardus*) and agile wallabies (*M. agilis agilis*) in the Top End of the NT (Dougall *et al.*, 2009). Prior to these fairly recent diagnoses, leishmaniasis was considered an exotic disease to Australia. It has not been seen in species other than macropods.

**Neospora caninum** is a protozoal parasite discovered in recent years. It is closely related to *T. gondii* and is similar to it in many respects. The dog (*Canis domesticus*) and the coyote (*Canis latrans*) are the definitive hosts (Dubey and Scharf, 2011). Neospora has been reported in a wide range of mammals (acting as intermediate hosts) including South American opossums and a Parma wallaby (*Macropus parma*) (Yai *et al.*, 2003, Dubey and Scharf, 2011, Cronstedt-Fell *et al.*, 2012). In Australia, it is speculated that native Australian marsupials may act as intermediate hosts as part of a sylvatic cycle for this pathogen. To date, there is little supportive evidence for this theory and understanding of the parasite transmission in marsupials is limited (Cronstedt-Fell *et al.*, 2012).

Dingoes and camp dogs in remote communities in the NT are seropositive for *Neospora* (King *et al.*, 2012). Infected dogs and dingoes pass only a small number of *Neospora* cysts in faeces for a short period of time. Cysts do not survive long in the environment and there is considerably less opportunity for environmental contamination with *Neospora* cysts, compared with *T. gondii* (Dubey and Schares, 2011).

Infection with *Neospora* can result in a range of outcomes, similar to infection with *T. gondii*. Information on *Neospora* infection in free-ranging Australian wildlife is limited; evidence for infection has only been found in macropods and in most cases was not associated with disease (Eymann *et al.*, 2006, Cronstedt-Fell *et al.*, 2012, Mayberry *et al.*, 2014). Although potentially of interest, there is no evidence that small mammal species are involved in *Neospora* epidemiology and there are limited diagnostic tests available for wildlife.

***Sarcocystis* spp.** have been reported in a variety of native mammals including rodents, bandicoots, brushtail possums and northern quolls. Although it is possible that disease may occur in some wildlife species, most infections do not appear to be pathological.

***Besnoitia* spp.** Individual species of this protozoal genus have been seen in Australian feral rodents. A syndrome associating *Besnoitia* spp. in grey kangaroos with disease is currently under investigation (WHA, 2011a). There is very little information about this pathogen in other Australian species.



## Appendix 5 - Scientific presentations and media interactions

A webpage was established for the project on the Conservation Medicine Program website at Murdoch University [www.murdoch.edu.au/School-of-Veterinary-and-Life-Sciences/Courses/Veterinary-postgraduate/Conservation-Medicine/Research/Investigating-the-role-of--disease-in-the-decline-of-small-mammal-species-in-the--Top-End-of-the-Northern-Territory/](http://www.murdoch.edu.au/School-of-Veterinary-and-Life-Sciences/Courses/Veterinary-postgraduate/Conservation-Medicine/Research/Investigating-the-role-of--disease-in-the-decline-of-small-mammal-species-in-the--Top-End-of-the-Northern-Territory/)

Scientific presentations were made at several national conferences over the course of the project and are summarised below.

***Is disease contributing to terrestrial mammal declines in Australia's Top End?*** A Reiss, K Warren, G Gillespie, B Jackson, L Skerratt, K Brennan and D Stokeld **Wildlife Disease Association Australasia annual conference**, the Grampians, Vic, 29 September – 4 October 2013; invited speaker for Symposium on Biodiversity and Disease Ecology.

***Where have all the mammals gone: is disease contributing to terrestrial mammal declines in Australia's Top End?*** A Reiss, K Warren, G Gillespie, B Jackson, L Skerratt, K Brennan and D Stokeld **Australian Veterinary Association (AVA) annual conference** May 2014, Perth, WA.

A media release was made by the AVA in relation to the presentation, with resulting phone interviews with journalists and an electronic story which circulated globally.

***Disease investigation in declining mammal populations in the Northern Territory*** A Reiss, G Gillespie, B Jackson, L Skerratt, D Stokeld, P Irwin, U Ryan, A Duarte, J Devlin & K Warren **Wildlife Disease Association Australasia annual conference**, Sept 2014, Tidbinbilla, ACT.

***Declining populations of small mammals in Australia's north: is disease the smoking gun?*** A Reiss, B Jackson, G Gillespie, D Stokeld, J Devlin, D Tompkins, R Hall, T Portas & K Warren **Wildlife Disease Association International Conference**, July 2015, Twin Waters, Qld.

During the course of the project Andrea Reiss undertook interviews with ABC radio journalists (Darwin local radio and ABC Radio National).

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